

NATURAL AND SYNTHETIC LONG-CHAIN FURANOID ACIDS

Rajendranath Chakrapani Wijesundra

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NATURAL AND SYNTHETIC
LONG-CHAIN FURANOID ACIDS

A thesis presented by
Rajendranath Chakrapani Wijesundera B.Sc. (Sri Lanka)
to the
University of St. Andrews
in application for
the degree of Doctor of Philosophy

September 1978



To my late mother

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Abstract

Fatty acids containing a furan ring system were recently discovered by North American Scientists in the liver and testicular lipids of northern pike. We developed an analytical procedure based on urea crystallization and argentation thin layer chromatography for the isolation of small quantities of these novel acids. Using this method a large number of fish lipids taken from both freshwater and marine habitats were examined. Our results show the wide occurrence of furanoid acids in fish at a low level (up to 5%). These unusual acids tend to concentrate in the cholesterol esters and appear to be absent from the phospholipids. Contrary to claims by the North American researchers we do not find a significant relation between the occurrence of these acids and the sex of the species or on whether the lipid is derived from a freshwater or marine source. We consider rather that the nutritional status of the animal is more significant. Cod liver oil usually contains furanoid acids at about 1% level but in two starved cod the proportion of these acids rose dramatically to 34 and 48% respectively.

We also examined four commercial fish meals but furanoid acids were not present in any of them.

Furanoid fatty acids were found to accumulate in the adipose tissue of rats given cod liver oil. No furanoid acids were demonstrable in comparable samples from rats given synthetic 10,13-furan acid.

A furanoid fatty acid has been isolated from Exocarpus cupressiformis seed oil. In two samples of this seed available to us we did not detect any furanoid acids but found 8-hydroxyoctadec-trans-11-en-9-ynoic acid (5%).

The methodology used in the isolation of furanoid acids also allowed the study of methyl-branched acids. Whilst confirming the wide distribution of three phytol-based acids in fish lipids we have also detected three other compounds which we believe are diunsaturated derivatives of 7,9-dimethylhexadecanoic, 7,9- and 9,11-dimethyloctadecanoic acids respectively.

The opportunity was also taken to study the more common acids of some fish species not previously examined. Besides confirming the well-known differences between the lipids of freshwater and marine origin, we find a significant difference between the fatty acids of male and female fish of freshwater origin, with the female containing more $n-3$ polyene acids and less monoene acids than does the male. The $n-3/n-6$ ratio is the most significant feature distinguishing freshwater from marine fish lipids.

The 10,13-, 9,12-, and 8,11-isomers of the C_{18} furanoid acids were prepared by one or more of the following methods.

(i) Pd(II)-catalysed cyclodehydrogenation of oxygen-containing unsaturated acids, (ii) dehydration of diepoxides and dehydrogenation of epoxy alkenes with propyl iodide-sodium iodide-dimethylsulphoxide, and (iii) dehydration of endoperoxides.

Synthesis of heterocyclic compounds by Pd(II)-promoted cyclization reactions is a fairly new development. The reactivity of various long-chain oxygenated esters with this unique reagent is discussed. The endoperoxide route is also novel and leads to speculation about the biosynthesis of natural furanoid acids.

Recognition and identification of long-chain furanoid acids by their chromatographic and spectroscopic behaviour is discussed. Mass spectrometry is a powerful method of structure determination of these substances. Some chemical reactions of long-chain furanoid acids are also described.

Declaration

I hereby declare that this thesis is a record of the results of my own experiments, that it is my own composition, and that it has not previously been presented in application for a higher degree.

The research was carried out in the Department of Chemistry, University of St. Andrews, under the supervision of Professor F.D. Gunstone, D.Sc., F.R.I.C.

Certificate

I hereby certify that Rajendranath Chakrapani Wijesundera has completed twelve terms of research work under my supervision, has fulfilled the conditions of the Resolution of the University Court 1967, No. 1 (St. Andrews) and that he is qualified to submit the accompanying thesis in application for the degree of Doctor of Philosophy.

Research Supervisor

Career

I entered the University of Sri Lanka (Peradeniya Campus) in October 1968 and graduated in November 1972 with Upper Second Class Honours in Chemistry.

After working for one year as a Temporary Assistant Lecturer in Chemistry at the same University, I joined the Ceylon Institute of Scientific and Industrial Research as a Research Officer. I worked in that capacity until October 1975, when I was awarded a Commonwealth Scholarship which has enabled me to follow the present course.

Acknowledgements

I wish to express my deepest gratitude to Professor F.D. Gunstone for the help, guidance, and encouragement he so readily gave me throughout this work. I am much indebted to him for interesting me in fatty acid chemistry.

I am also grateful to Dr. Charles Scrimgeour for his help in and out of the laboratory. I should also like to thank the technical staff of the Chemistry Department for their friendly and efficient service and Dr. A. McGill of the Torry Research Station (Aberdeen) for GC-MS facilities.

The following people kindly helped me obtain fish lipid samples: Dr. D.B.C. Scott (Zoology Department), Dr. I.A. Johnston (Physiology Department), Professor M.S. Laverack (Gatty Marine Laboratory), Mr. R.S. Cross (Marfleet Refining Company, Hull), and Dr. D. Ross (Torry Research Station, Aberdeen). Dr. M. Keith (Unilever Research) provided the fish meal samples and Dr. R. Goldsack (CSIRO, Australia) supplied the Exocarpus cupressiformis seeds. Dr. ^GJ. Goodlad (Biochemistry Department) and Mr. T. Oliver (Animal House) helped me to carry out the feeding experiments.

I must also thank the Commonwealth Scholarship Commission for enabling me to pursue this course and the Ceylon Institute of Scientific and Industrial Research for granting me study leave.

Last but not least, I thank Miss Fiona Sutherland for typing this thesis.

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Abbreviations

Fatty acids are reported in shorthand by showing the number of carbon atoms followed by a colon and a figure denoting the number of multiple bonds. The nature of unsaturation (shown by the letters a, c, and t indicating acetylenic, cis olefinic, and trans olefinic groups respectively) and its position relative to the carboxyl group are given in parenthesis.

e.g. 18:1(9c) is oleic acid
 18:2(12a9c) is crepenynic acid

Other functional groups are represented by the appropriate symbol prefixing the number of carbon atoms in the acid.

e.g. 12OH-18:1(9c) ricinoleic acid

Δ gives the position of the double bond counting from the carboxyl-end and n-x shows its location from the methyl-end where x is the position of the first double bond counting from the methyl-end.

e.g. 18:2(n-6) is 18:2(Δ 9, Δ 12).

Ag ⁺ TLC	Argentation thin layer chromatography
b.p.	Boiling point
CE	Cholesterol esters
CMR	Carbon ¹³ nuclear magnetic resonance. A typical CMR signal is reported as 14.09(32) - the first figure is the chemical shift in ppm and the figure in parenthesis is the peak height.
DEGS	Diethylene glycol succinate
DMF	Dimethylformamide

DMSO	Dimethylsulphoxide
ECL	Equivalent chain length
ϵ	Molar extinction coefficient
F	Furanoid. Symbols F_1 , F_2 etc. denote the furanoid acids commonly occurring in the fish lipids, the structures of which are shown in Table 2.
10,13-furan	10,13-epoxyoctadeca-10,12-dienoic acid (or methyl ester)
9,12-furan and 8,11-furan	have similar meaning
GC-MS	Gas chromatography - mass spectrometry
GLC	Gas liquid chromatography
IR	Infrared. Expressions in parenthesis after the absorption frequency indicate the shape and intensity of the peaks respectively. The symbols have the following meanings. (b) broad, (f) fine (s) strong, (m) medium, (w) weak
M	Molecular ion
mp	Melting point
MS	Mass spectrometry. The m/e value is followed by two figures in parenthesis the first of which indicates the ion fragment and the second its intensity expressed as a percentage of the base peak. Unless stated otherwise, the peak at m/e 74 should be assumed to result from McLafferty rearrangement involving the ester carbonyl group.

P ₁₆	Methyl 4,8,12-trimethyltridecanoate [4,8,12-TMTD]
P ₁₉	Methyl 2,6,10,14-tetramethylpentadecanoate [2,6,10,14-TMPD (pristanic)]
P ₂₀	Methyl 3,7,11,15-tetramethylhexadecanoate [3,7,11,15-TMHD (phytanic)]
PE	Denotes a mixture of petroleum (bp 40-60°) and diethyl ether. When a number appears after the abbreviation, it shows the percentage by volume of ether in petrol.
PL	Phospholipid
PMR	Proton magnetic resonance (¹ H nuclear magnetic resonance). A typical PMR signal is represented as 0.88(t, 3H, CH ₃ CH ₂ -), meaning a triplet at 0.88 ppm downfield from tetramethylsilane as internal standard with an integral indicating three protons. The triplet is attributed to methyl group and is split due to the neighbouring methylene group. The symbols used to describe the multiplicity of the signals are: s = singlet, d = doublet, t = triplet, m = multiplet.
Prep.	Preparative
R _f	Retention index (TLC)
TG	Triacylglycerol
THF	Tetrahydrofuran
THP	Tetrahydropyran
TLC	Thin layer chromatography
TMS	Trimethylsilyl
UV	Ultraviolet

Abstract

Fatty acids containing a furan ring system were recently discovered by North American Scientists in the liver and testicular lipids of northern pike. We developed an analytical procedure based on urea crystallization and argentation thin layer chromatography for the isolation of small quantities of these novel acids. Using this method a large number of fish lipids taken from both freshwater and marine habitats were examined. Our results show the wide occurrence of furanoid acids in fish lipids at a low level (up to 5%). These unusual acids tend to concentrate in the cholesterol esters and appear to be absent from the phospholipids. Contrary to claims by the North American researchers we do not find a significant relation between the occurrence of these acids and the sex of the species or on whether the lipid is derived from a freshwater or marine source. We consider rather that the nutritional status of the animal is more significant. Cod liver oil usually contains furanoid acids at about 1% level but in two starved cod the proportion of these acids rose dramatically to 34 and 48% respectively.

We also examined four commercial fish meals but furanoid acids were not present in any of them.

Furanoid fatty acids were found to accumulate in the adipose tissue of rats given cod liver oil. No furanoid acids were demonstrable in comparable samples from rats given synthetic 10,13-furan acid.

A furanoid fatty acid has been isolated from Exocarpus cupressiformis seed oil. In two samples of this seed available to us we did not detect any furanoid acids but found 8-hydroxyoctadec-trans-11-en-9-ynoic acid (5%).

The methodology used in the isolation of furanoid acids also allowed the study of methyl-branched acids. Whilst confirming the wide distribution of three phytol-based acids in fish lipids we have also detected three other compounds which we believe are diunsaturated derivatives of 7,9-dimethylhexadecanoic, 7,9- and 9,11-dimethyloctadecanoic acids respectively.

The opportunity was also taken to study the more common acids of some fish species not previously examined. Besides confirming the well-known differences between the lipids of freshwater and marine origin, we find a significant difference between the fatty acids of male and female fish of freshwater origin, with the female containing more n-3 polyene acids and less monoene acids than does the male. The n-3/n-6 ratio is the most significant feature distinguishing freshwater from marine fish lipids.

The 10,13-, 9,12-, and 8,11-isomers of the C₁₈ furanoid acids were prepared by one or more of the following methods. (i) Pd(II)-catalysed cyclodehydrogenation^d of oxygen-containing unsaturated acids, (ii) dehydration^d of diepoxides and dehydrogenation^d of epoxy alkenes with

propyl iodide-sodium iodide-dimethylsulphoxide, and
(iii) dehydration of endoperoxides.

Synthesis of heterocyclic compounds by Pd(II)-promoted cyclization reactions is a fairly new development. The reactivity of various long-chain oxygenated esters with this unique reagent is discussed. The endoperoxide route is also novel and leads to speculation about the biosynthesis of natural furanoid acids.

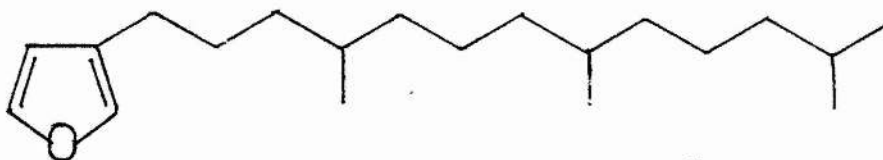
Recognition and identification of long-chain furanoid acids by their chromatographic and spectroscopic behaviour is discussed. Mass spectrometry is a powerful method of structure determination of these substances. Some chemical reactions of long-chain furanoid acids are also described.

Section I: OCCURRENCE

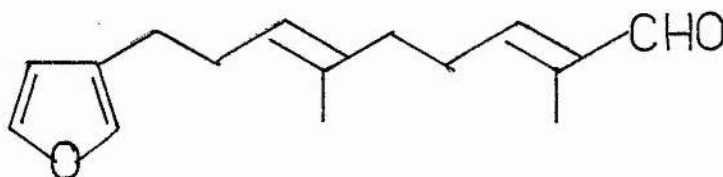
INTRODUCTION

Chemical compounds containing a furan ring system are widely distributed in nature and possess a diversity of structural features. Several long-chain furanoid substances have been isolated from both plant and animal sources and although they are most commonly based on a mono-substituted 3-furan¹⁻⁵, products based on mono-substituted 2-furan and di-substituted 2,5-furan are known too⁶⁻⁸.

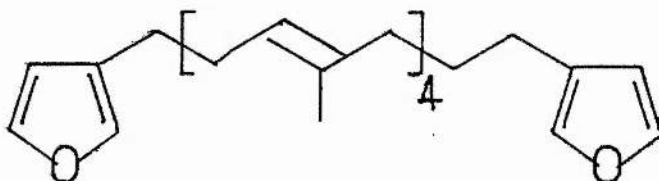
Table 1: Structure and Origin of Some Long-chain Furanoid Natural Products



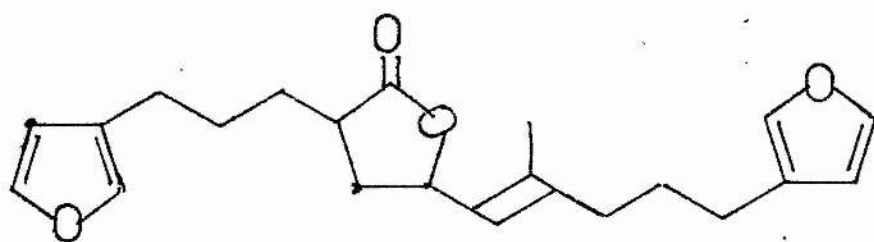
Phytofuran (cigarette smoke)¹



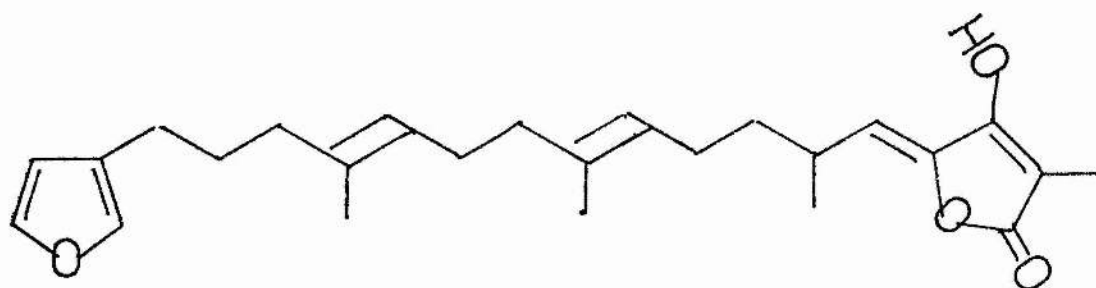
Torreyal (Torreyia nucifera)²



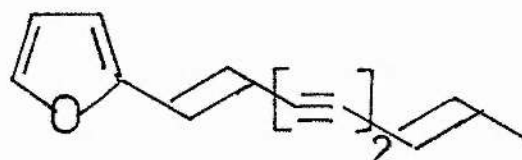
Difluvospinosulin
(Ivcinia spinosula)³



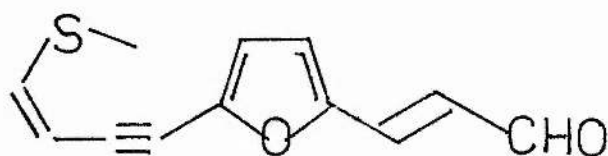
(Spongia nitens)⁴



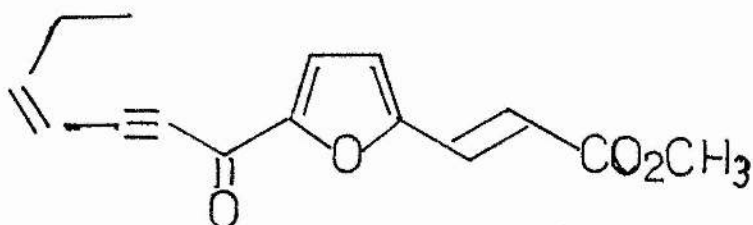
Strobilinin (Ircinia strobilina)⁵



Atractylodin (Atractylodes mandshur)⁶



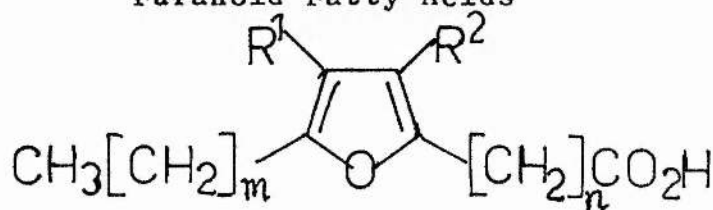
(Chrysanthem coronarium)⁷



Wyerone (Vicia faba)⁸

The first fatty acid containing a furan ring system was reported by Morris et.al.⁹ in 1966, when they isolated the C₁₈ acid (F_E) from Exocarpus cupressiformis seed oil. Until that time no heterocyclic fatty acids were known except for epoxy acids. Its discovery aroused considerable interest at the time and Smith¹⁰ reviewing the rare fatty acids in plants wrote that "the furanoid fatty acid characterized by Morris et.al. is probably the most exotic of the unusual fatty acids yet discovered".

Table 2: Structures of Some Naturally Occurring Furanoid Fatty Acids



	F ₁	F ₂	F ₃	F ₄	F ₅	F ₆	F ₇	F ₈	F ₉	F _E
R ¹	Me	H	Me	Me	H	Me	Me	H	Me	H
R ²	Me	Me	Me	Me	Me	Me	Me	Me	Me	H
m	2	4	4	2	4	4	2	4	4	5
n	8	8	8	10	10	10	12	12	12	7

No other furanoid fatty acids were discovered until almost ten years later when Glass and coworkers^{11,12} reported the presence of a series of them F₁-F₆ and F₈, F₉ in several tissues of the northern pike (Esox lucius). These acids are of two series with m=2 or 4 and have chain lengths ranging from C₁₆ to C₂₂ and, the C₂₀ acid F₆ being predominant. In contrast to the acid isolated from

Exocarpus cupressiformis seed oil, the pike acids all have a methyl substituent at the 3-position of the furan ring and the 4-position contains either a hydrogen atom or a methyl group.

Glass et.al. found these novel acids primarily in the liver and testes lipids from northern pike and observed a wide seasonal fluctuation of their amounts. For example, at the spawning time of northern pike in early spring, the new acids were <5% of all acyl moieties in liver lipids, but reached levels of 65% in testes lipids. In the late summer, they were found in amounts up to 25% of the acids in the total liver lipids whereas the amounts in the testes fell to 30%. Furanoid acids were also found in several other male freshwater fish. Although Glass et.al. detected low levels of these in some female fish, they claimed that furanoid acids are predominantly a male characteristic.

These workers reported that in the testes lipids of northern pike, the furanoid acids are concentrated in the triacylglycerols and absent from the phospholipids. In the liver lipids, they were concentrated in the cholesterol esters and to a lesser degree, in the triacylglycerols. In the late summer, the furanoid acids represented more than 90% of the acids bound in cholesterol esters in the liver lipids. This preferential esterification to cholesterol and the fluctuation in amounts in the testes which reach a maximum at the time of reproduction and a minimum after that period, suggested a physiological role of the furanoid acids in fish. Substitution of a 5-membered ring by an alkyl and a carboxy-alkyl chain is

reminiscent of prostaglandin structures. Indeed, physiological activity resembling that of some prostaglandins has been claimed for several synthetic furan-2-octanoates having a variety of aliphatic substituents in position 5 of the ring¹³.

The principal purpose of the work described in this thesis was to discover how widely furanoid fatty acids occur in fish lipids and to study their chemistry. A random selection of fish lipids taken from both freshwater and marine habitats were examined and although the primary objective was to investigate the furanoid acids, the methodology used also permitted the study of unusual methyl-branched acids. The opportunity was also taken to review the more common acids present in some species not previously examined.

To facilitate the discussion of the results obtained from the analytical work, there follows a discussion of the available information on the fatty acid composition of lipids of fish and other aquatic animals.

Component Acids of Lipids from Aquatic Animals

Analyses of lipids from aquatic sources are covered by a vast literature stemming from the early work of Lovern¹⁴. Because of greater abundance and commercial value fish oils, naturally, have been the subject of many studies¹⁵⁻³¹ and the results have been reviewed^{32,33}.

The fatty acids derived from lipids of fish and other aquatic animals are numerous and more complex than those obtained from depot fats of land animals or from

seeds of vegetable plants and this difference has been emphasised in recent years by the discovery of many fatty acids of novel structure from such sources. They include, in addition to the already mentioned furanoid acids, (1) methyl-branched acids, (2) odd-chain length acids, (3) nonmethylene-interrupted dienoic acids (4) C_{24} - C_{30} polyenoic acids, (5) other unusual polyene acids, and (6) acids containing trans unsaturation. These unusual acids are normally minor components of the lipid though their amounts could become significant under special circumstances.

(A) The Major Acids

(i) Structure

It has been reported that the major component acids of lipids of aquatic origin are characterized by the following features³⁴:








(a) They usually exceed twenty in number and vary in chain length from C_{14} to C_{24} .

(b) Saturated acids are mainly palmitic (16:0, 15-20%) accompanied usually by myristic (14:0) and stearic (18:0).

(c) Monoene acids (35-60%), whilst predominantly C_{18} (mainly Δ^9 and Δ^{11}), also include C_{16} (Δ^9), C_{20} (Δ^9 and Δ^{11}), and C_{22} (Δ^{11} and Δ^{13}) members.

(d) The remaining acids are mainly polyenes of the $n-6$ (18:2 to 22:5) and $n-3$ (18:3 to 22:6) families: the latter group predominate and most lipids from aquatic sources contain 20:5 and 22:6 ($n-3$) acids as major components.

Table 3: Names and Structures of Some Fatty Acids Commonly Occurring in Lipids of Aquatic Origin

Structure	Name	Symbol
Saturated Acids		
	Tetradecanoic acid (Myristic acid)	14:0
	Hexadecanoic acid (Palmitic acid)	16:0
	Octadecanoic acid (Stearic acid)	18:0
Monounsaturated Acids		
	Hexadec-9-enoic acid (Palmitoleic acid)	16:1(n-)
	Octadec-9-enoic acid (Oleic acid)	18:1(n-)
	Eicos-11-enoic acid (Gadoleic acid)	20:1(n-)
	Docos-11-enoic acid (Cetoleic acid)	22:1(n-)

Structure

Name

Symbol

n-6 Acids

Octadeca-9,12-dienoic acid
(Linoleic acid)

18:2(n-6)



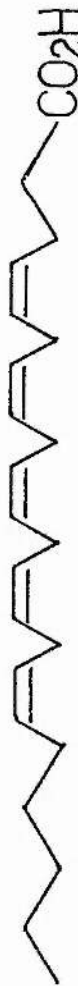
Octadeca-6,9,12-trienoic
(γ-Linolenic acid)

18:3(n-6)



Eicosa-5,8,11,14-tetraenoic
acid
(Arachidonic acid)

20:4(n-6)



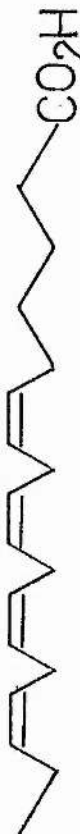
Docosa-4,7,10,13,16-pentaenoic
acid

22:5(n-6)

n-3 Acids

Octadeca-9,12,15-trienoic acid
(α-Linolenic acid)

18:3(n-3)



Octadeca-6,9,12,15-tetraenoic acid

18:4(n-3)



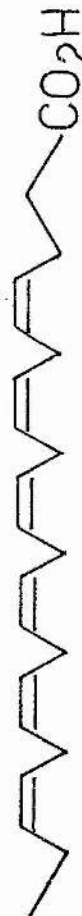
Eicosa-5,8,11,14,17-pentaenoic
acid

20:5(n-3)



Docosa-7,10,13,16,19-pentaenoic
acid

22:5(n-3)



Docosa-4,7,10,13,16,19-hexaenoic
acid

22:6(n-3)

Although these statements provide a useful background with which actual results can be compared, they oversimplify a complex situation. The nature as well as the relative proportions of the component acids of lipid from aquatic animals are variable and are greatly influenced by many external factors. In the ensuing account the origin of these fatty acids and their regulation by environmental factors are considered.

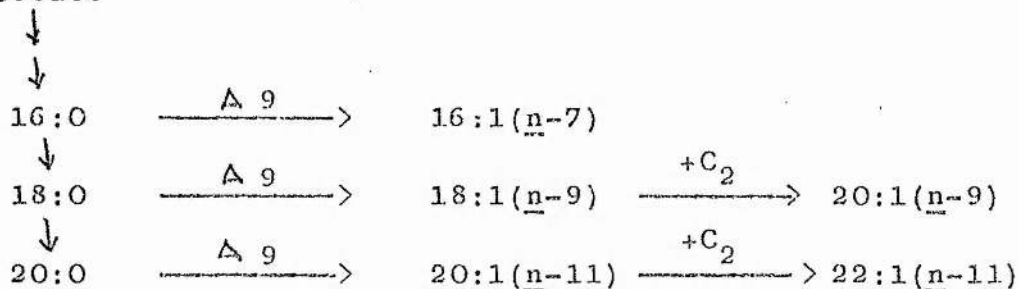
(ii) Origin

The major component acids of fish lipids can be divided into those synthesized directly from C_2 precursors (endogenous) and those derived from the diet (exogenous).

(a) Endogenous Acids:

Like all other life forms, fish synthesize saturated fatty acids by the de novo pathway utilizing acetate. The product is mainly palmitate which may then undergo a series of chain-elongation and desaturation steps.

Acetate



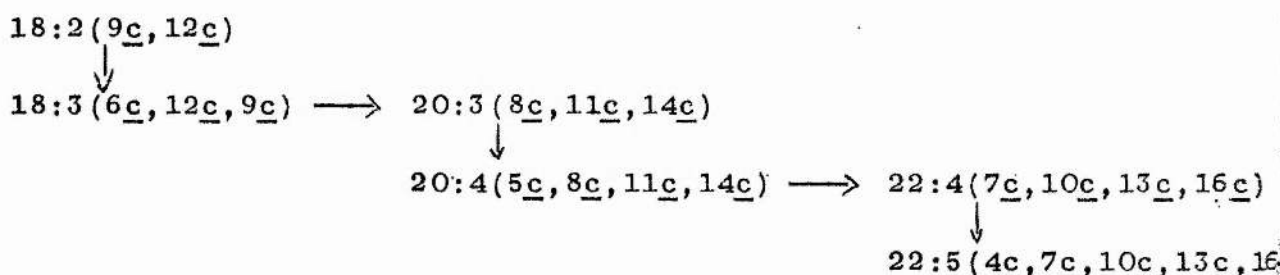
It is noteworthy that the $22:1(\underline{n-11})$ fatty acid is universally preferred in fish lipids to $22:1(\underline{n-9})$ (erucic acid) which could be formed by chain elongation of the abundant $20:1(\underline{n-9})$ acid. It is possible that, $22:1(\underline{n-11})$ is formed

by a mechanism whereby 20:0 fatty acid is desaturated to 20:1(n-11) by a Δ^9 desaturase followed by chain-elongation of the latter by the conventional mechanisms^{35,36}.

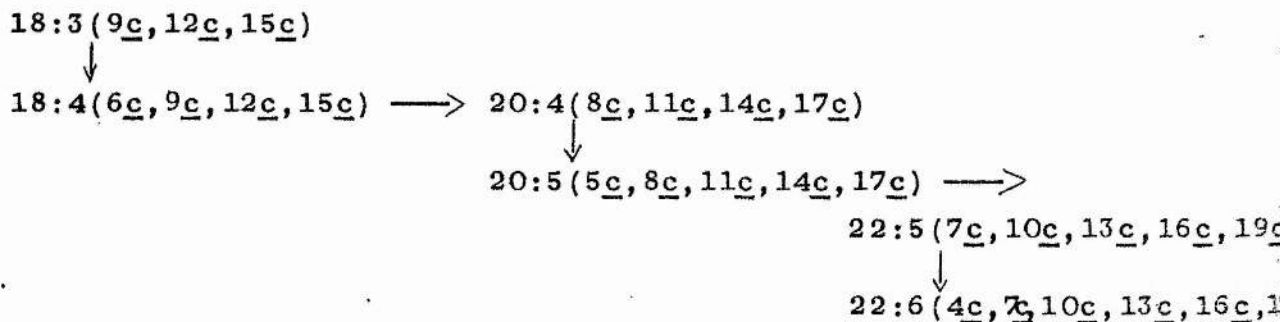
(b) Exogenous Acids

Unlike plants, animal enzymes normally introduce new double bonds between an existing double bond and the carboxyl group³⁷. It is significant that fish do not contain a desaturase which can introduce a double bond beyond the Δ^9 position of the monounsaturated acids obtained de novo, and the n-6 and n-3 polyene acids which are abundant in fish lipids must originate in the diet. Some or all the polyunsaturated fatty acids essential to the fish may be already present in the diet or may be obtained by metabolism of dietary linoleic and linolenic acids. The following biosynthetic pathways have been proposed.

n-6 family



n-3 family



Studies carried out on freshwater and euryhaline fish indicate that the ability of these species to elongate and desaturate dietary fatty acids is similar to that of birds and mammals. Thus when rainbow trout were fed diets containing oleic, linoleic or α -linolenic acids, substantial quantities of 20:3(n -9), 22:5(n -6), or 22:6(n -3), respectively, appeared in the lipids of different tissues³⁸. However, recent experiments with marine flat fish like plaice³⁹ and turbot⁴⁰ have revealed that these species lack the ability to chain-elongate and desaturate oleic, linoleic or α -linolenic acids. Owen et.al.⁴⁰ fed groups of turbot with radioactive oleate, linoleate and α -linolenate and showed that these were incorporated without modification into the tissue lipids. This confirmed that turbot cannot desaturate and chain-elongate C_{18} fatty acids.

Marine fish other than flat fish also appear incapable of further desaturating and chain elongating dietary α -linolenic acid. Thus the red bream (chrysophrys major) grew poorly on diets containing 3-4% α -linolenic acid and converted food inefficiently whereas diets containing long-chain polyunsaturated fatty acids of the n -3 series at a level of 2% led to rapid growth and good feed conversion^{41,42}.

In a recent review, Cowey and Sargent⁴³ have speculated that these fish have lost or never acquired the ability to form polyunsaturated fatty acids from C_{18} precursors because, being carnivores, they obtained plenty of polyunsaturated acids in their food. Thus the ability to convert, for example, 18:3(n -3) to 22:6(n -3) would have

little selection value. They pointed out that the ability to convert C_{18} acids to polyunsaturated acids (in this case 18:2(n-6) to 20:4(n-6) is also lacking in the extreme carnivores cat (Felis catus)⁴⁴ and lion (Panthera leo)⁴⁵. It seems that here is an instance of biochemical evolution along parallel lines between divergent forms in quite separate environments. It appears therefore, that in the case of a few fish, at least, the total requirement of polyunsaturated fatty acids are obtained preformed in the diet.

Recently, Crawford et.al.⁴⁶ showed that the fatty acids of the dolphin (Tursiops truncatus) bear a much closer resemblance to those of land animals than to those of other marine vertebrates. The food chain of the dolphin provides a vast preponderance of the n-3 fatty acids compared to n-6, while the land food chain provides a more even balance between the two series. Yet, the results showed that the dolphin has a significantly lower n-3/n-6 ratio than that of marine vertebrates. The ancestors of the dolphin evolved on the land with the terrestrial n-3/n-6 balance and it appears that the dolphin fatty acids reflect its evolutionary origin rather than its present environment.

We have seen that certain fish obtain their polyunsaturated fatty acids entirely from the diet, and in others the origin is more complex. To add to this complexity, aquatic animals unlike their terrestrial counterparts are intimately exposed to fluctuating

environmental conditions such as oxygen concentration, temperature, and pressure (depth). Such variables involve the modification and regulation of the lipid and component fatty acids of the animal.

(iii) Environmental Influences

(a) Temperature

Temperature is a major environmental factor well established as causing changes in the fatty acid composition of fish lipids. In general, a decrease in environmental temperature induces an increased degree of unsaturation of the tissue fatty acids. This effect has been observed in goldfish muscle⁴⁷, brain^{48,49}, and intestinal⁵⁰ lipids; rainbow trout⁵¹, as well as other fish^{52,53}. Although most of the experimental work in this field has been done on freshwater fish, the phenomenon also applies to marine fish⁵⁴.

An increase in unsaturated fatty acids at low temperatures may result from an increase in the amounts of phospholipids rich in these acids. That this was not the case was demonstrated by Root⁴⁹, who showed that change in environmental temperature does not influence the amount of the major phospholipids found in goldfish brain. Brenner et. al.⁵⁵ investigating the effect of environmental temperature on the activity of liver microsomal desaturases from Pimelodus maculatus found that fish kept at 15° had higher desaturation and elongation activity than those kept at 30°. This increase in desaturation activity,

evoked at relatively low environmental temperatures, fully compensated for the decrease in specific reaction rate with falling temperature characteristic of enzyme-catalysed reactions. They were, however, unable to correlate this data with the proportion of polyunsaturated acids present in the liver microsomes at the two temperatures, as there were actually less polyunsaturated acids at the lower temperature. Similar discrepancies have been reported by other workers⁴⁷.

Poikilothermic animals, such as fish, do not have a constant body temperature. The adaptation of the physio-chemical properties of their membranes to ever-changing temperatures, therefore, has considerable survival value⁵⁶. The increase in fatty acid unsaturation with decrease in temperature may be a means of adjusting membrane viscosity within the range necessary for metabolic processes⁵³. Roots⁴⁹ has suggested that the modification of fatty acid composition assists in the maintenance of proper membrane fluidity and permeability for efficient functioning of the nervous system.

(b) Effect of Pressure (Depth)

Like temperature, the pressure too has an influence on the fatty acid composition of the lipids from aquatic animals but at present, there is not sufficient data to allow a precise statement about its relationship to the fatty acid pattern. Living in deep sea has meant adaptation to darkness, low temperatures and special food supply in addition to high hydrostatic pressure⁵⁷. Earlier

it was thought that the pressure has less significant effect on deep sea life than temperature⁵⁸. Subsequent studies^{59,60} have shown that the relationship between temperature and pressure is indeed more complex. Despite the considerable work in deep sea biology and biochemistry neither the effect of hydrostatic pressure on biomembrane structure nor the membrane lipid fatty acid composition as a function of depth in marine water column is known⁶¹.

One of the few attempts to correlate fatty acid composition with depth in the ocean is that of Lewis⁶². He analysed fatty acids of a variety of fish species from different depths down to 4400 m and found that concentration of medium-chain saturated acids (16:0 and 18:0) and long-chain unsaturated acids (C_{20} and C_{22}) decreased with depth, whereas the levels of oleic acid (18:1) increased with depth. These findings were supported by the recent work of a Japanese group⁶³, who examined the polyenoic fatty acid contents of liver neutral lipids in six species of fish. These observations must reflect to some extent at least, the known presence of wax esters rich in oleic acid in certain midwater species⁶⁴.

(c) Influence of Sex and Season

We have seen that fatty acid composition of fish lipids is influenced by environmental factors such as the temperature and pressure. We have also seen that it is dependent on the dietary intake of the animal. Most fish, especially those living in the sea, experience wide seasonal fluctuations in food supplies. If one samples

the plankton at different times of the year, he would find contrasts between spring, summer, autumn and winter in the sea almost as striking as those in the vegetation on the land. These seasonal changes in plankton have a profound effect on the lives of many fish.

As the environmental factors vary depending on the season, it is to be expected that the fatty acid composition also changes accordingly. Seasonal changes are extremely complicated however, as the effects embrace not only the environmental influences but also those of maturity and spawning⁶⁵. To quote Jacquot⁶⁶ "The significance of the seasonal variations is complex and it is almost impossible to distinguish surely between the effects of the many factors which play a part".

Nevertheless, several papers have documented changes in fatty acid composition in relation to season. DeWitt⁶⁷ observed that the liver lipids of the cod (Gadus callarias) show an increase in polyunsaturated and monoenoic acids during the winter and summer months. An abrupt decline in these acids took place during the March spawning season, and no variation in saturated fatty acids was evident through the annual cycle. Jangaard and coworkers⁶⁸ investigating the seasonal changes that occurred in the fatty acids of cod (Gadus morhua), found that the fatty acids from the flesh showed no significant changes with respect to either sex or season. However, the hepatic lipids of female fish showed increasing amounts of 20:1 and 22:1 acids in late summer and fall, while other acids did not vary significantly. The male fish on the

other hand did not exhibit similar seasonal variations. Gruger et.al.¹⁶, examining two mullet-oil samples taken in the months of July and December demonstrated the striking differences that are possible for a single species caught during different seasons. The fish were caught in the same general area but the December samples contained half as much 16:2 and four times as much 22:6 as was found in the July samples.

Unlike most terrestrial animals, the majority of fish experience severe depletion of lipids and other body constituents for a part of every year in their lives. This depletion may be due either to scarcity of food at certain periods of the year or be the effect of maturation and spawning. The production of eggs or sperm always depletes a fish but in certain fish such as salmon, ascending the rivers to spawn, the condition may be exaggerated by a concurrent abstention from food⁶⁵. Such depletion can effect marked changes in the fatty acid composition of the lipid. For example, Lovern⁶⁹ observed that the fatty acids of the body lipid from mature Clupea harengus are more saturated than that of fish at an earlier stage of maturation. Presumably this is caused by preferential utilization of unsaturated acids, though a change in body size or the nature of the diet could also be important. Iverson⁷⁰ having determined the fatty acid profiles of prime and spent salmon concluded that the long-chain monounsaturated acids are preferentially utilized by the migrating salmon. The real situation, however, may be more complex than that as apparent from the work of Pjleger⁷¹ who noted a

pronounced difference in liver triglyceride synthesis between ocean pre-spawning and river post-spawning pink salmon (Onchorhynchus gorbuscha). After entering fresh-water and spawning these fish had virtually lost the ability to synthesize triglyceride in the liver but the ability to synthesize cholesterol esters had increased.

(B) The Minor Acids

(i) Methyl-branched acids

Three main types of methyl-branched acids occur in fish lipids and each will be considered separately.

(a) Iso- and anteiso-acids

Iso and anteiso acids carry a methyl group on the penultimate and on the antepenultimate position of the carbon chain respectively and constitute by far the most widely distributed group of branched-chain fatty acids⁷². The C₅ compound iso valeric acid belongs to this group and has long been known as a constituent of whale oil^{73,74}. Occurrence of longer-chain members in a fish oil was first demonstrated in 1956 by Morice and Shorland, who isolated C₁₅ and C₁₇ iso- and anteiso-acids from shark liver oil⁷⁵. Later, Ackman et.al. found small amounts of C₁₄-C₁₈ iso-acids and C₁₅ and C₁₇ anteiso-acids in tissues of several species of fish taken from both marine⁷⁶ and freshwater⁷⁷ habitats. Monounsaturated derivatives of iso and anteiso acids⁷⁸ have recently been found as minor constituents among the fatty acids of a marine diatomaceous ooze⁷⁹. Their presence in fish lipids, however, has not been reported.

Biosynthetically, these acids originate from leucine, valine or isoleucine⁷². With the exception of iso-14:0 and iso-16:0, iso- and anteiso-acids are absent from marine plants and bacteria is the likely source of these in fish lipids though a proportion may be generated by animal biosynthesis⁷⁷.

(b) Acids with one methyl branching in the middle of the chain

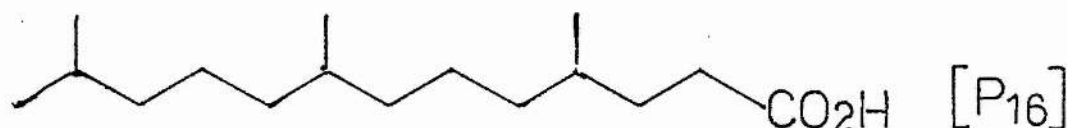
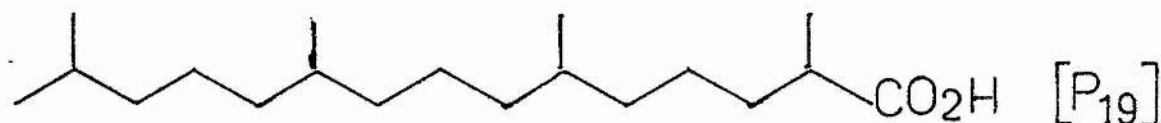
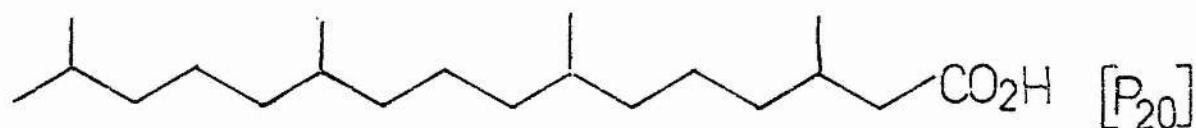
Although the occurrence in fish lipids of saturated acids with a single methyl-branch is rare or the amounts often insignificant, several of their unsaturated derivatives have been discovered in fish. Sano⁸⁰, in 1967, isolated 7-methylhexadec-6-enoic acid from the oils of fin whales and sperm whales. Gupta⁷² showed later that this acid is accompanied in whale oil by a series of methyl-hexadecanoic acids with the methyl substituent located at C-6 through C-15, 8-, 9-, 10- and 14-methylheptadecanoic acids, and 12-methylpentadecanoic acid. More recently, Ackman et.al.⁸¹ showed the presence of the isomeric 7-methylhexadec-7-enoic acid in the liver oil of ocean sun fish Mola mola in relatively high quantities (2-3%). Trace amounts of this acid were also found in the lipids of jellyfish⁸², spadefish⁸³, marine turtles⁸⁴ as well as a sea anemone⁸⁵.

Most marine lipids probably contain some 7-methyl-hexadec-7-enoic acid as evidenced by the almost universal occurrence of minor (<1%) amounts of the methyl ester of 7-methylhexadecanoic acid in hydrogenated ester samples⁸². Although Sano⁸⁰ did not detect this acid when he discovered

the $\Delta 6$ isomer in whale oils, Ackman et.al. who examined sperm whale oils taken from northern as well as southern hemispheres found both 7-methylhexadec-6-enoic acid (0.23-0.68%) and 7-methylhexadec-7-enoic acid (0.37-1.37%) to be present in varying proportions in the different oils. Small amounts of 5-methyltetradec-4-enoic acid were also present⁸⁶. The origin and significance of this group of acids remains obscure.

(c) Multiple Branched Acids with Isoprenoid Structure

A saturated C_{20} multiple-branched acid was first isolated by Hansen and Shorland^{87,88} and subsequently identified by Sonneveld et.al.⁸⁹ as 3,7,11,15-tetramethylhexadecanoic acid. This compound (P_{20}), named phytanic acid, has since been reported from a large variety of sources ranging from human and animal tissues to geological sediments^{72,90,91}.



In addition to phytanic acid a number of other isoprenoid fatty acids have been discussed in various sources. Thus, 2,6,10,14-tetramethylpentadecanoic acid (P_{19} , pristanic acid) was first identified by Hansen in butter fat^{92,93} and sheep fat⁹⁴ and was subsequently isolated from a fish oil by Sen Gupta and Peters⁹⁵. Phytanic and pristanic acid together with the C_{16} homologue, 4,8,12-trimethyltridecanoic acid are found commonly in many fish lipids and the widespread occurrence of this group in marine organisms⁹⁶⁻¹⁰⁶ as well as in freshwater fish⁷⁷ is well documented.

It has long been known that phytanic acid is derived from phytol (3,7,11,15-tetramethylhexadec-trans-2-en-1-ol) which is the alcohol moiety of chlorophyll, and it is very likely that phytol is also the precursor for the biosynthesis of phytanic acid in marine organisms¹⁰¹⁻¹⁰⁴. The C_{19} (pristanic) and C_{16} acids are believed to arise from catabolism of phytanic acid⁹⁰.

(ii) Odd-Chain Acids

Small amounts of fatty acids containing odd-numbered chains commonly occur in the lipids of ruminants and have been reported recently from a seed oil¹⁰⁷. They are also found in fish lipids and generally account for 1-2% of the total fatty acids^{108,67,16,76}. However, mullet-oil has been shown to contain 10-25% of odd-numbered acids (both saturated and unsaturated) depending on the area and season of catch^{16,109} and more recently Ackman has reported up to 10% of C_{15} , C_{17} and C_{19} acids in the lipids of smelt

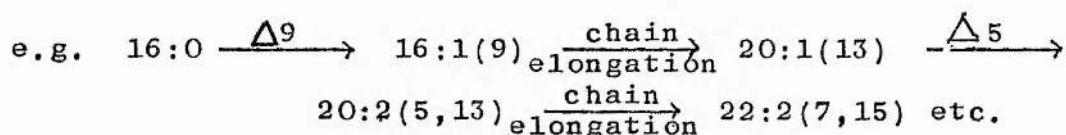
(Osmerus mordax)¹¹⁰. The exceptionally high levels of odd-chain fatty acids in smelt was linked to its diet comprising of the amphipod Pontoporeia femorata (Kröyer) which was found to contain as much as 50% of odd-numbered acids^{111,112}.

(iii) Nonmethylene-Interrupted Dienoic Acids

In natural polyunsaturated fatty acids, double bonds are separated by a single methylene group. This characteristic methylene-interrupted pattern prevails in the majority of cases, but exceptions do occur. For example, some plant species have been reported to contain significant quantities of dienoic acids whose double bonds are interrupted by more than one methylene group^{10,113-119}. This group of acids generally referred to as 'nonmethylene-interrupted dienoic acids' (NMID) have also been found as trace components in the lipids of terrestrial animals^{120,121} often as the result of certain dietary restrictions^{122,123}. Recently minute quantities of NMID acids have been isolated from several marine organisms. For example, Paradis and Ackman¹²⁴ reported two homologous series of C₂₀ and C₂₂ NMID acids in the lipids of the American oyster Crassostrea virginica and identified them as 20:2(5,13); 22:2(7,15); and 20:2(5,11); 22:2(7,13) respectively. The periwinkle (Littorina littorea), moon snail (Lunatia triseriata), sand shrimp (Crangon septemspinosus), and the atlantic sturgeon (Acipenser oxyrhynchus) are also thought to contain the same major NMID acids as the oyster¹²⁵, but the lipid of the white shrimp (Penaeus setiferus) has been shown to contain

additional isomers; 20:2(7,11 and 7,13), 21:2(7,13) and 22:2(7,13; 7,15; 9,13; 9,15; and 7,17)¹²⁶.

The NMID acids are possibly formed from saturated acids through the operation of a Δ^9 and Δ^5 desaturase at an appropriate stage in the chain-elongation sequence.



Ackman and Paradis have suggested that one of the several invertebrate phyla may be responsible for these acids in marine organisms. They also suggested that the distribution of NMID fatty acids is based on relationships within the food web and that these unusual acids could be much more useful than common fatty acids in examining food-web relationships¹²⁷.

(iv). C₂₄-C₃₀ Polyene Acids

The Δ^{15} C₂₄ monoene has long been known in fish lipids⁷⁴. Presence of small amounts of polyenoic C₂₄ acids in these lipids was first observed more than forty years ago by several Japanese workers¹²⁸⁻¹³¹.

In 1964, Ackman et.al. identified 24:5(9,12,15,18,21) and 24:6(6,9,12,15,18,21) in the liver oil of cod (Gadus callarias)¹³² and the oil of pilchard (Sardinops sagax)¹³³.

Later Link and Karinkanta²⁴ showed the presence of several C₂₄-C₂₈ methylene-interrupted polyenoic acids in the flesh lipids of the Baltic herring (Clupea harengus var. membranus). The C₂₄ acids consisted of tetraenes, pentaenes and hexaenes

and the C_{26} acids of pentaenes and hexaenes all of which had $n-3$ Structure. The C_{28} acid, however, was a $n-6$ heptaene and these unusual acids together accounted for 5.3% of the total fatty acids.

More recently Litchfield and co-workers demonstrated the presence of unusually high levels of $C_{24}-C_{30}$ non-methylene-interrupted polyunsaturated acids in the lipids of several marine sponges. First they found them in Microciona prolifera whose lipids contained 26:2(5,9) [14%] and 26:3(5,9,19) [31%]^{134,135}. Earlier, Bergmann and Swift¹³⁶ had observed the presence of 26:1(9) and 26:2(17,20) in two other marine sponges and Litchfield et.al., attempting to find out whether these ultra long-chain acids are characteristic of all sponges or are limited to a few species, examined twenty different genera of the Demospongiae which is the most numerous of the three taxonomic classes in the phylum Porifera (sponges). All of them contained exceptionally high levels (34-79%) of $C_{24}-C_{30}$ polyenoic acids. They concluded that these acids are characteristic of the Demospongiae and named them "demospongic acids"¹³⁷. One of these sponges, Xestospongia halichondroides, contained 26:1(17) [21%], 26:2(5,9) [27%], and 28:3(5,9,19) [13%]¹³⁷. Of these only the 26:1(17) acid has been reported from a fish oil²⁴.

Morales and Litchfield¹³⁸ have shown that the sponge Microciona prolifera possesses a very active fatty acid chain-elongation system that produces $C_{24}-C_{28}$ acids from normal chain length precursors, and it seems likely that

similar biosynthetic pathways occur in the other sponges.

(v) Other Unusual Polyene Acids

A heneicosapentaenoic acid (21:5) has been reported from a variety of marine species such as copepods^{139,140}, shrimps^{106,141}, molluscs¹⁴², and fish^{102,143} and in higher animals such as marine turtles, seals, dolphins, and large whales^{85,144-146}. Most of these identifications were tentative and were based on GLC retention times and supplemental information such as resistance to urea adduct formation and conversion to 21:0 by hydrogenation. The positions of the double bonds in the molecule had never been established.

Recently Mayzaud and Ackman isolated a 21:5 acid from seal oil and identified it as the n-3 member by mass spectrometry (as pyrrolidine derivative) and by comparison with an authentic sample. In seal oils the 21:5 acid is more obvious than in fish oils but even so represents only 0.5% of the total acids. The n-3 structure is unusual for an odd-chain polyenoic acid and Mayzaud et.al. suggested that it may be derived from α -oxidation of 22:5(n-3) although ω -oxidation of the 21:6 hydrocarbon common in marine algae is also a possibility¹⁴⁷.

Moderate to high levels (4-23%) of 18:5(n-3) acid has been found in several species of photosynthetic marine dinoflagellates¹⁴⁸. Various species of herbivorous copepods as well as contemporary carnivorous chaetognaths living in the same environment present traces of this acid¹⁴⁹. On moving up the food chain, however, the quantity of 18:5(n-3)

decreases¹⁴⁹ and its presence in fish or other higher animals has not been reported.

(vi) Acids Containing trans Unsaturation

A fatty acid containing trans unsaturation, viz 16:1(6t) has been reported in the lipids of several marine organisms including marine turtles^{85,150,151}, coelenterates^{152,153} and fish^{82,154}. Spadefish (Chaetodipterus faber) contains 4.6%¹⁵⁴ of this unusual acid while in coelenterates and marine turtles its amount varied from 0.1-3%. The presence of this acid in many different marine species of diverse origin would suggest its deposition from a common food source¹⁵⁰. Because coelenterates appear to be such a common food source of this fatty acid in certain fish and turtles, the substantial presence of 16:1(6t) in sunfish and spadefish could be reflection of a high dietary intake of such organisms¹⁵⁴.

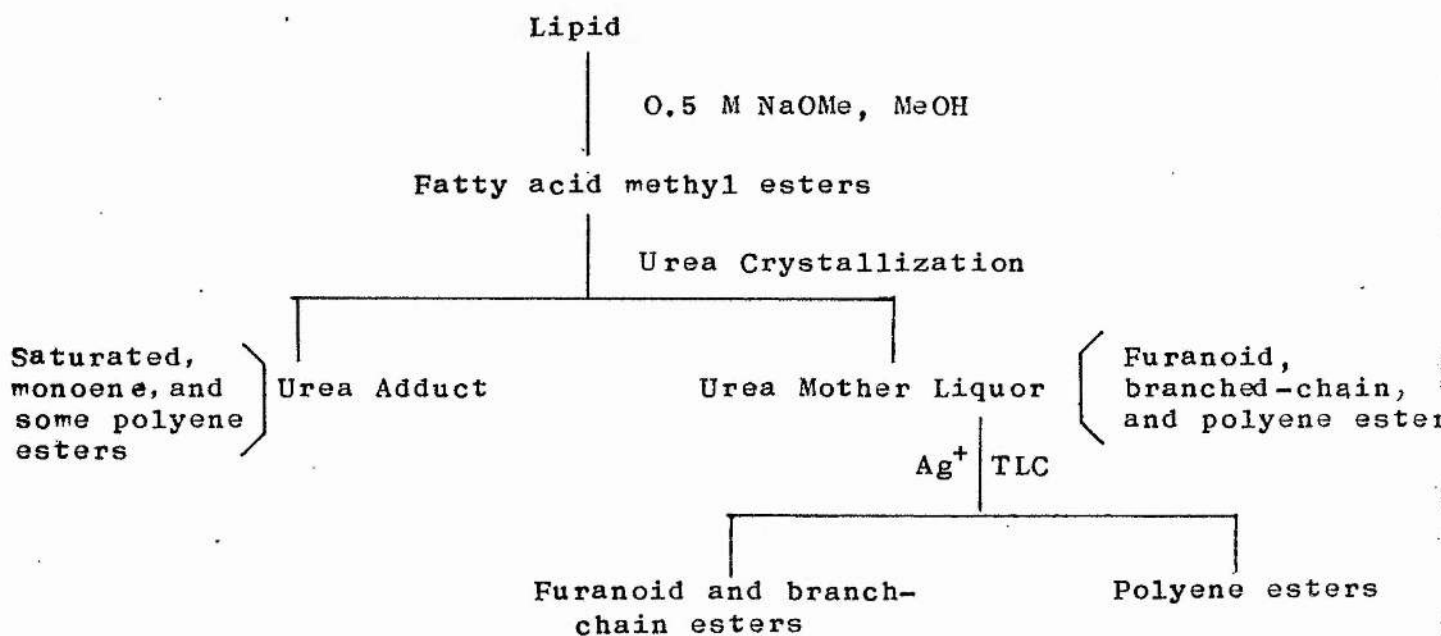
RESULTS AND DISCUSSION

1. FURANOID ACIDS

(i) Isolation

A procedure based on urea crystallization and Ag^+ TLC was developed for the isolation of furanoid acids of lipids from fish and other natural sources. The lipid was transesterified with sodium methoxide in methanol (acid catalysis was avoided because of the lability of furans towards acids, see Section III), and the methyl esters were crystallized from methanol in the presence of 5 times their weight of urea. This removed all of the saturated and monounsaturated esters and part of the polyunsaturated esters as urea adducts which crystallized from the methanol solution. Methyl esters of branched-chain and furanoid fatty acids (if any) were retained in solution together with the remainder of the polyene esters (Fig 1-4)

Scheme 1 Isolation of Furanoid Esters from Fish Lipids.



The esters recovered from the mother liquor were then submitted to preparative Ag^+ TLC. Furanoid esters are not retarded appreciably by silver ion and migrate like saturated esters. This permitted a ready separation of furanoid esters from the polyene esters and also from traces of any monoene esters that might be retained in the mother liquor.

Ethanollic 2',7'-dichlorofluorescein is commonly employed as a non-destructive spray reagent for the detection of fatty acid methyl esters on TLC plates. The esters usually appear as yellow bands when viewed under UV light. Furanoid compounds, however, do not show up so well and they are not easily detected with this spray reagent unless appreciable quantities are present. If small amounts of saturated and monounsaturated esters are still present in the liquor (from urea crystallization), the furanoid esters (usually comprising of 1-5% of the liquor) can be seen as a bluish-black band between two yellow bands representing the former compounds. For most purposes it is, of course, desirable to isolate F esters free from saturated or monoene esters and therefore an alternative method of detection was developed.

When sprayed with ethanolic phosphomolybdic acid fish furanoid esters show up as dark brown spots at room temperature. An authentic sample of F esters extracted from a fish oil was run along one edge of the TLC plate at the same time as the mixed esters from the urea-mother liquor were being chromatographed. With only the edge strip exposed,

the plate was sprayed with phosphomolybdic acid solution when a dark brown spot developed at room temperature, within 1-2 min, denoting the location of the furan band. Spraying the entire plate with ethanolic dichlorofluorescein located the polyene ester bands and also complemented the location of the furanoid band by the phosphomolybdic spray.

Glass and coworkers adopted a somewhat different approach to the isolation of furanoid esters from pike lipids^{11,12}. They found that unsaturated fatty esters could be selectively hydrogenated in the presence of the tri- and tetra-substituted furanoid esters occurring in pike lipids. The total methyl esters were submitted to controlled hydrogenation and the product was crystallized with five times their weight of urea. The mother liquor now contained F esters and branched-chain esters which were isolated without further purification.

Glass et.al. carried out the hydrogenation using PtO_2 as catalyst and under these conditions only the common fatty acid methyl esters were hydrogenated. Our studies have confirmed their results but we find that F esters not containing a methyl substituent on the ring are markedly affected under the same conditions (see Section III). A non-methylated F acid has been isolated from a seed oil⁹ and although such compounds have not been reported so far from fish oils use of Glass's method would preclude their isolation, if present. Moreover, small quantities of unsaturated derivatives of furanoid esters have been observed both by Glass et.al. and us. Isolation of these,

Isolation of furanoid esters from cod liver oil
by urea crystallization followed by Ag^+ TLC.

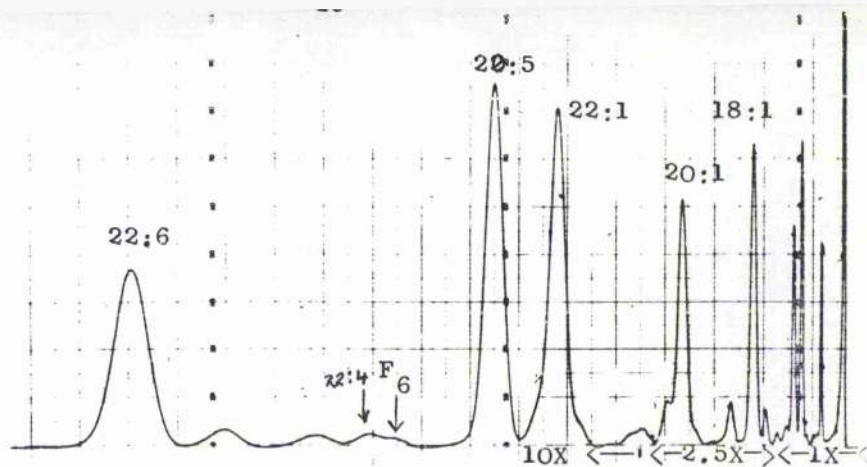


Fig. 1: Total Esters

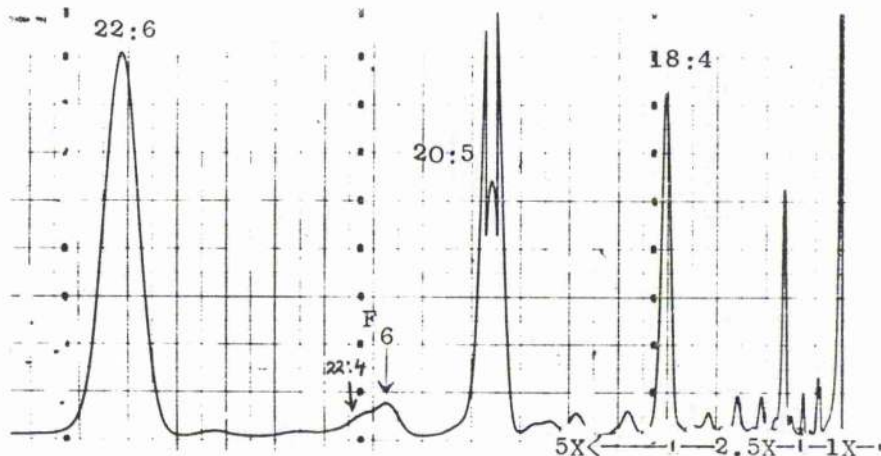


Fig. 2: Urea Mother Liquor

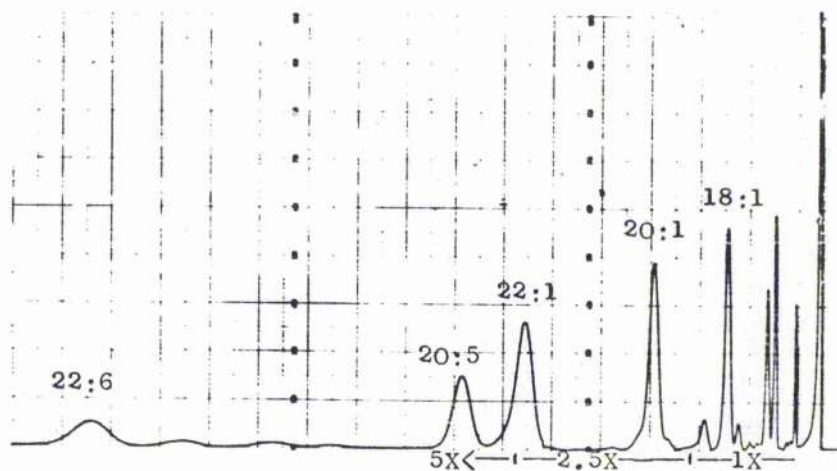


Fig. 3: Urea Adduct

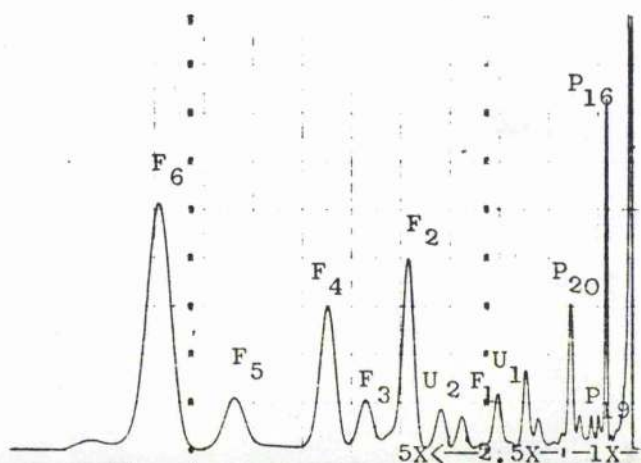


Fig. 4: Furan Band

also requires a procedure which does not involve hydrogenation.

Figures 1-4 illustrate the distribution of the cod liver oil methyl esters between the adduct and mother liquor during urea crystallization and the isolation of furanoid esters by Ag^+ TLC of the liquor. This pattern of fractionation is typical of most fish oils.

Normally F acids are present in fish lipids in exceedingly small quantities and during GLC of the total esters they are masked by the peaks due to the major components. F_6 , which is the principal member of the furanoid acids commonly occurring in fish lipids, can be sometimes seen in the total esters as a shoulder on the peak representing $22:4(\underline{n}-6)$. (Fig. 1).

Treatment of the total esters with urea in quantities five times their weight ensures that all of the saturated and monoene esters are removed by crystallization (Fig. 2). This operation also removes part of $22:4(\underline{n}-6)$ and the presence of F_6 becomes apparent at this stage (Fig. 3).

The other F acids (F_1-F_5) are not visible at this stage because they are masked by the polyene peaks. For example, F_5 overlaps with $20:5(\underline{n}-3)$ and F_3 with $20:4(\underline{n}-6)$. It should also be noted that F_4 and F_2 overlap with $22:1$ and $20:1$ respectively. This, however, does not constitute a problem because both the monoenes are completely removed by urea crystallization and are usually absent from the liquor. No polyene or monoene peaks occur in the region where F_2 appears and this peak can be normally detected

in the mother liquor (Fig. 3).

In the present study the furanoid esters (mainly F_1 - F_7) were isolated together with branched-chain esters (mainly P_{16} , P_{19} , P_{20}) and are collectively referred to as 'the furan band' (Fig. 4). On Ag^+ TLC, the three phytol-based esters P_{16} , P_{19} , P_{20} migrate ahead of, but very close to the furanoid esters and the separation of these two classes from each other at the same time as they are being separated from the polyene esters requires extreme care. The furanoid esters could be purified by rechromatography of the furan band on silica (TLC) using a fairly non-polar solvent system such as PE5. Table 4 illustrates the separation obtained when 100 mg of a mixture of F esters and P esters were applied on a single TLC plate. More satisfactorily separations can be achieved by using less material per plate.

Table 4: Separation of Furanoid Esters from Phytol-based Esters ^a

<u>Identification</u>	<u>Mixture^b</u>	<u>Band 1^c</u>	<u>Band 2</u>
P_1	8.8	18.9	4.1
P_2	7.9	19.5	0.1
P_3	18.8	41.8	1.5
F_1	1.2	-	2.7
F_2	5.9	0.8	11.5
F_3	2.2	0.2	4.5
F_4	8.5	1.8	16.2
F_5	4.5	1.6	7.2
F_6	39.0	15.4	47.0
F_7	0.9	-	1.0
U_1	1.9	-	3.4
U_2	0.4	-	0.8

- a Chromatography on layers of silica (1 mm thick) using P85 as developing solvent (100 mg of mixture on one plate)
- b This extract was prepared from cod liver oil by urea crystallization followed by Ag^+ column chromatography
- c Refers to the upper band [38% of the mixture (by weight)]

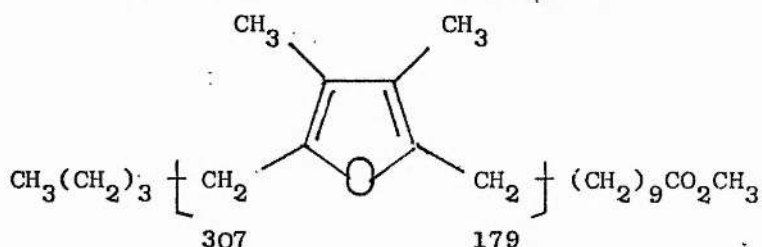
For analytical purposes, however, such separations were not necessary because the phytol-based esters eluted fairly quickly during GLC and did not interfere with the analysis of furanoid esters (see Fig. 4).

(ii) Characterization

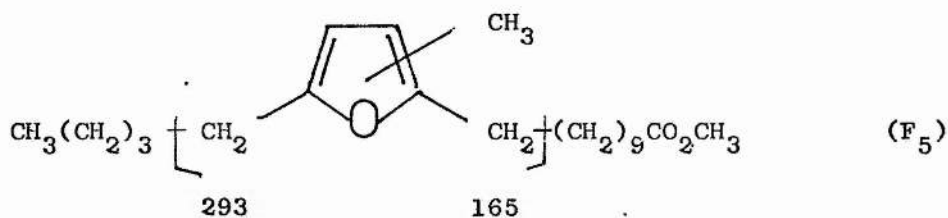
The identification of the furanoid esters isolated from fish lipids was based mainly on their GLC behaviour and mass spectrometry. Furanoid esters exhibit very characteristic mass spectra (see Section III) and their structures were confirmed by this method. In a large-scale experiment dogfish liver oil was fractionated by urea crystallization followed by Ag^+ column chromatography [Section 1 (v)]. The fractions containing furanoid esters were investigated thoroughly by GC-MS and structures were assigned to each GC peak. In subsequent analyses, the common components F_1 - F_7 occurring in fish lipids were identified by their ECL values. This was possible because the furan band often gave a characteristic finger print with F_6 almost always being the biggest followed usually

by F_4 and F_2 (Fig. 4).

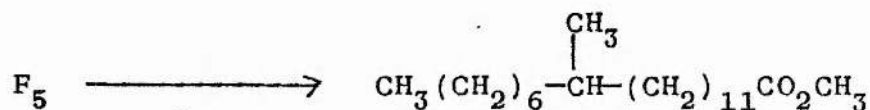
As already mentioned mass spectroscopy is a versatile method of characterizing furanoid fatty acids. Unlike that of normal fatty acids, the mass spectra of furanoid fatty acid methyl esters are simple and generally display four high intensity peaks by means of which their structures can be determined. For example, the high resolution mass spectrum of F_6 shows an intense molecular ion of $m/e = 364.2958^{11}$ which corresponds to a molecular formula of $C_{22}H_{40}O_3$. It also showed intense peaks at $m/e = 307$, 179, and 123: the first two arising from β -cleavage on either side of the ring and the latter from



double β -cleavage. These four mass spectral signals determine the chain-length, position of the ring, and the number of methyl substituents on the ring. However, when the ring contains only one methyl substituent such as in F_5 , the mass spectrum is not sufficient to determine the position of the methyl group unambiguously. An intense peak at $m/e = 109$ shows that this compound contains a mono-methylated furan-ring and the peaks at 350, 293, and 165 show that it contains a C_{20} -chain of $n-6$ structure. The methyl substituent could be on either 3- or 4-position of the ring.

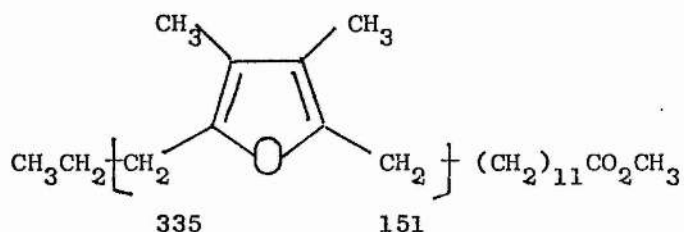


Glass and coworkers¹² resolved this problem by chemical conversion of a mixture of F₁-F₆ to their corresponding saturated acyclic esters. The mass spectra of the resulting methyl-branched compounds (obtained by GC-MS) unequivocally fixed the position of the methyl groups. For example, the derivative of F₅ was identified



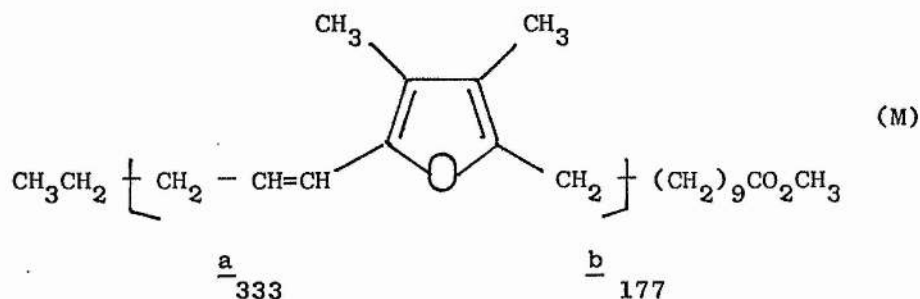
as the methyl ester of 13-methyleicosanoic acid proving that F₅ contains the methyl substituent at the 3-position of the ring. In the structural assignments reported in this thesis such a 3-substitution pattern is assumed for all mono-methylated F acids.

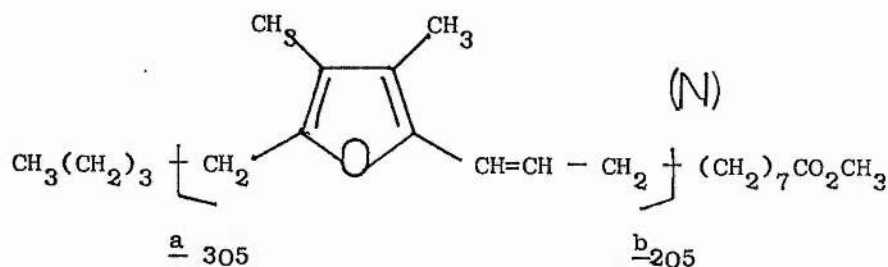
We identified nine furanoid acids (F₁-F₉) in fish lipids and their structures are shown in Table 2. Of these, F₇ which is isomeric with F₆ is reported for the first time. This new member occurs consistently in the furan band of most fish lipids as a minor constituent (1-2%) and was identified mainly by its mass spectrum.



The molecular ion at m/e 364 was confirmed by a fragment ion at 31 mass units less. A major ion at m/e 123 showed that the ring is di-methylated and the base peak at m/e 151 and the peak at m/e 335 unequivocally fixed the hetero atom at 14,17-positions of the chain.

Evidence for the presence of unsaturated furanoid esters was also found. GC-MS of a furanoid extract from dogfish liver oil revealed two peaks of BCL 26.6 and 26.9 [fraction 3, see Section I (v)] each of which had the molecular ions at m/e 362. Thus they were mono-unsaturated derivatives of F_6 and major fragment ion pairs at m/e 333, 177 and m/e 305, 205 suggested that at



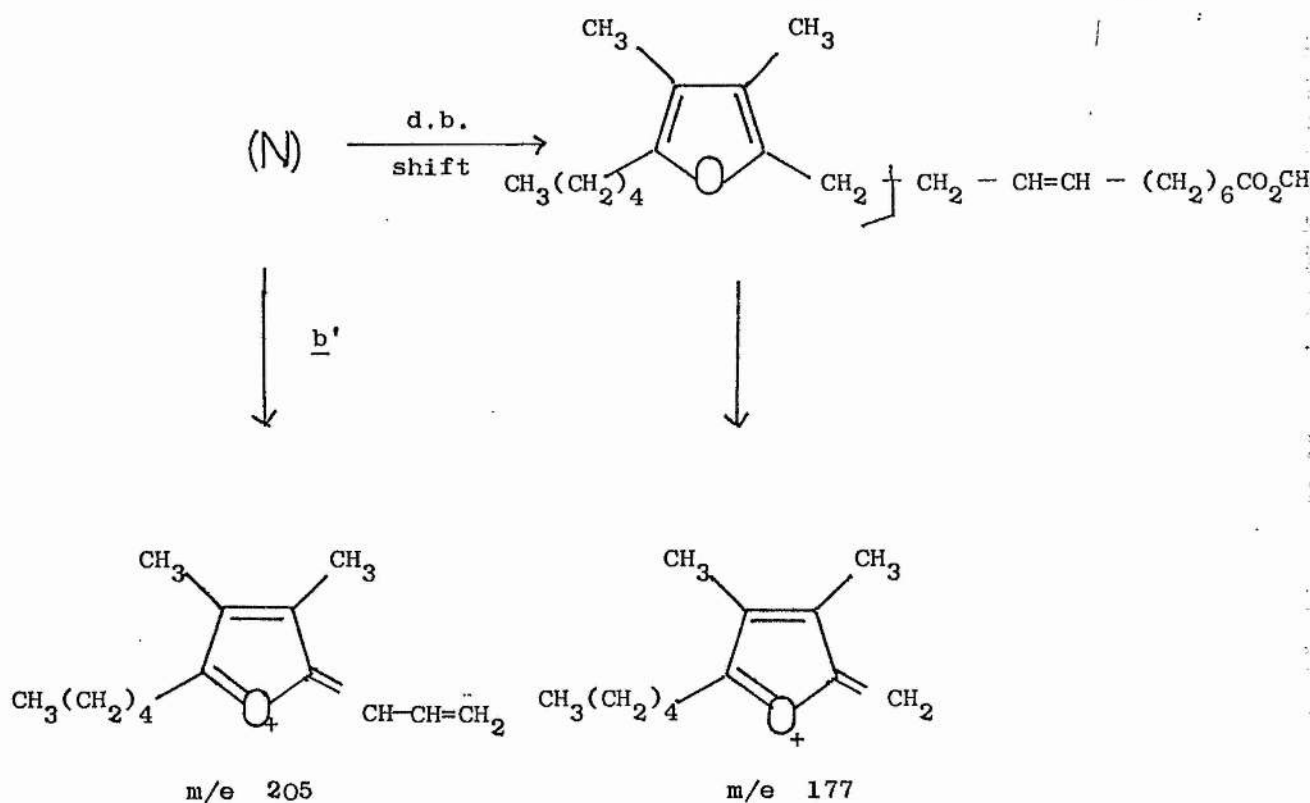


least two isomers were present with the double bond on either side of the ring. (Structures M and N). Fragment ions representing both isomers were present in either peak and although the relative intensities of the major fragment ions (Table 5) were biased towards structure (M) for the former peak and towards (N) for the latter, such assignments could be only tentative in view of the

Table 5: Some Major Fragment Ions in the Mass Spectra of Two Unsaturated F Esters

Structure	Fragment Ion	m/e	<u>intensity</u>	
			<u>ECL 26.6</u>	<u>ECL 26.9</u>
M	a	333	39	27
	b	177	79	57
N	a'	305	13	24
	b'	205	37	65

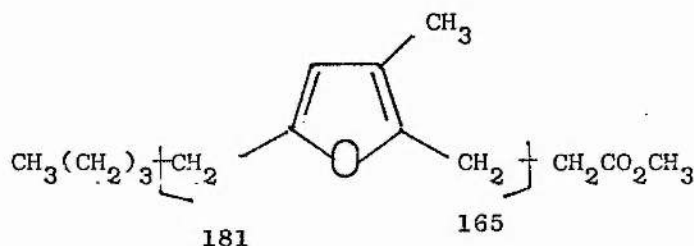
fact that double bond shifts occur during mass spectrometry. The fragment ion at m/e 177, for instance, could have arisen from isomers due to such double bond migration.



These unsaturated derivatives were consistently present in fish lipids which contained F acids. Their amounts, however, were exceedingly small and their isolation is difficult unless a sizeable sample is available. Their presence in the furan band was ignored except in a few special cases where F acids were the major components in the lipid [starved cod, Section 1 (ix)]. In this case, Ag^+ TLC of a concentrate of F esters prepared by urea fractionation showed an additional band migrating closely behind the normal furan band. This band contained four peaks which are not observed in the normal furan band to any large extent. Two of these peaks were the same as the

unsaturated esters found in dogfish liver oil. The other two had the same retention times as F_8 and F_9 and were not identified.

We have also mass spectral evidence for a C_{12} mono-methylated F acid. GC-MS of F esters isolated from dogfish liver oil [fraction 5, Section 1 (v)] showed a peak (ECL 15.5) whose molecular ion was at m/e 238. Its mass spectrum contained a peak at m/e 109 which indicated a mono-methylated furan ring and the presence of the base peak at m/e 165 and another intense fragment ion at m/e 181 provided strong evidence for the following structure.



(iii) Gas Chromatography

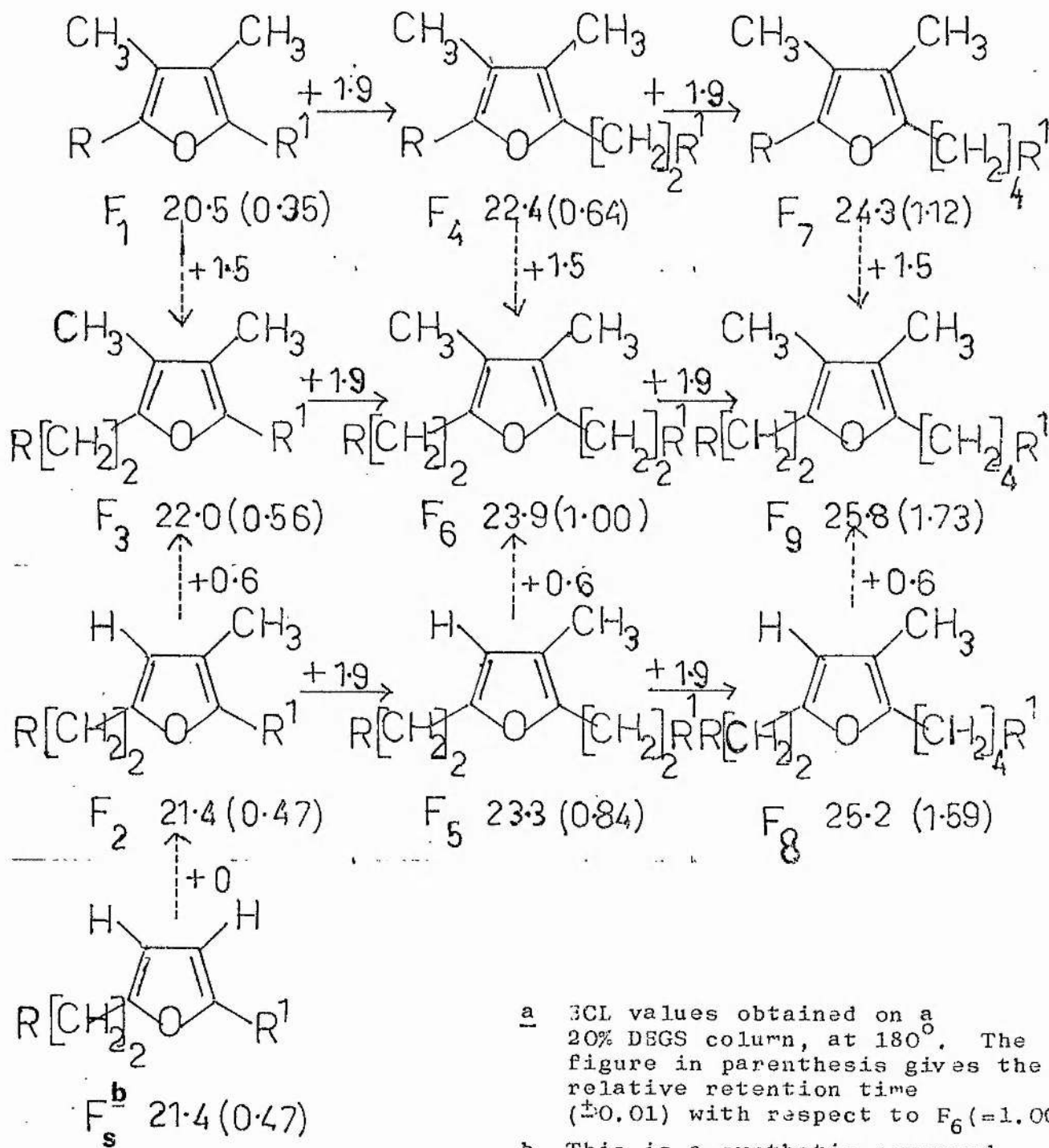
Of the nine furanoid esters identified in fish lipids F_1 - F_7 eluted within 1 h when chromatographed on a DEGS column. F_8 and F_9 are usually small relative to other members of the series and this fact coupled with their longer retention times makes them difficult to detect on DEGS columns. Newer packing materials such as SP 222 PS and especially SP 2300 give much lower retention times and the entire series F_1 - F_9 including the unsaturated members eluted from these columns within a reasonable time (~ 40 min).

F_1 - F_7 were well separated from each other on all packings used. F_8 and F_9 , though separated from each other appeared to overlap with some of the unsaturated members. F_1 was immediately followed by U_2 and baseline separation between these two was not always obtained. Also a minor peak appeared just after F_2 and although the presence of this component was obvious on neither DEGS nor SP 222 PS columns, a satisfactory separation was obtained when chromatographed on a 20% EGGS-X column.

Polarity of the stationary phase had little effect on the ECL's of the F esters. Thus change from SP 2300 to more polar SP 2340 or SP 222 PS gave virtually the same ECL values. This consistency reflects the fact that the F esters and the saturated fatty acid methyl esters with respect to which ECL values are calculated have similar polarity.

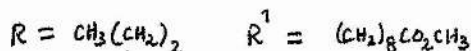
Although the new stationary phase SP 222 PS was valuable for detection of longer-chain and unsaturated derivatives of the F esters, reproducibility of ECL values on this column was not particularly good. SP 2300 and SP 2340 gave more consistent ECL values and these two columns had advantage over SP 222 PS in that the latter was thermally unstable and was extremely sensitive to moisture. As far as reproducibility of ECL values are concerned DEGS was the best stationary phase and on this column almost identical ECL values were obtained for the F esters over a 3 month period. The analyses reported on DEGS were carried out on the same column over a period of 8 months and the few

Table 6: ECL Values^a of Some of the Naturally Occurring Furanoid Methyl Esters.



^a ECL values obtained on a 20% DEGS column, at 180°. The figure in parenthesis gives the relative retention time (± 0.01) with respect to F₆ (=1.00)

^b This is a synthetic compound.



variations in ECL's observed were presumably due to aging of the column.

The slight variations in the ECL values were not a great problem in the identification of the F esters for such changes were the same for all members. Thus the relative retention time of F esters with respect to F_6 ($=1.00$) were remarkably constant (± 0.01). These values were particularly useful for the unambiguous identification of F esters in samples where the characteristic furan finger print was not observed.

Table 6 shows the ECL values and relative retention times (RRt) with respect to F_6 ($=1.00$) of several furanoid methyl esters. The relative retention times of some common polyene esters with respect to F_6 ($=1.00$), and also with respect to $20:5(\underline{n}-3)$ ($=1.00$) are given in Table 7.

Table 7: Relative Retention Times of Some Common Esters with Respect to $F_6 = 1.00$ and $20:5(\underline{n}-3) = 1.00$

	ECL	RRt ^a	RRt ^b
P_3	17.0	0.13	0.17
$18:4(\underline{n}-3)$	20.7	0.39	0.50
$20:4(\underline{n}-6)$	22.0	0.58	0.76
$20:5(\underline{n}-3)$	22.9	0.77	1.00
$22:6(\underline{n}-3)$	25.3	1.58	2.06
F_6	23.8	1.00	1.30

^a Relative retention time w.r.t. $F_6 = 1.00$

^b Relative retention time w.r.t. $20:5(\underline{n}-3) = 1.00$

Comparison of the ECL value of the 10,13-furan (C_{18}) with that of methyl stearate shows that the furan ring has an increment of about 3 units (2.7-3.4) over that of the corresponding saturated acyclic ester. This holds only when the ring is contained in the central portion of the chain and as Lie Ken Jie et.al.^{156,157} have recently reported the ECL's of the isomers within one chain length increase gradually when the furan system is moved in either direction from the centre.

The synthetic C_{18} 10,13-furan and its mono-methyl analogue F_2 were not separable on any of the polar stationary phases used. Thus it appears that furanoid esters with only one methyl substituent on the ring are not easily separated by GLC from those containing none.

Addition of two methylene groups to the carboxyl-end of the chain increases the ECL by 1.9 whereas similar addition to the methyl-end gives an increment of only 1.5. Introduction of a second methyl substituent onto the furan ring causes an increase in ECL of 0.6 (Table 6).

(iv) Quantitation

Since the furanoid acids were normally only minor components of fish lipids their amounts could not be calculated directly from GLC of the total esters. After fractionation by urea crystallization, however, F_6 which is the dominant member of the series appears in the GLC trace of the mother liquor. If p and q are the percentage composition

of F_6 in the liquor and the furan band respectively, then the content of furanoid esters in the total (f) is given by

$$\underline{f} = \underline{n} \times \underline{p}/\underline{q} \times \underline{m}/100 \%,$$

where, \underline{n} is the % of furanoid esters in the furan band and \underline{m} is the % of the mother liquor.

The recovery of esters from the urea fractionation was frequently incomplete and the proportion of adduct and mother liquor was best determined on the basis of the proportion of the major components in these two fractions and in the total esters. For example, let the percentage composition of any given ester in the total esters, mother liquor and adduct be \underline{x} , \underline{y} , \underline{z} respectively. Also suppose the weight percent of the liquor and adduct are \underline{m} and \underline{a} respectively.

$$\text{Then, } \underline{a} + \underline{m} = 100$$

$$\text{and also, } 100\underline{x} = \underline{a}\underline{z} + \underline{m}\underline{y}$$

$$\text{hence, } 100\underline{x} = \underline{a}\underline{z} + (100-\underline{a})\underline{y}$$

$$\underline{a} = \frac{(\underline{x}-\underline{y})}{\frac{\underline{z}-\underline{y}}{(\underline{y}-\underline{z})}} \times 100$$

Use of any of the major acids such as 16:0, 18:1, 20:1, 22:1, 20:5, 22:6 etc., in any one case, gives similar values for \underline{a} and the average of them was usually taken as the weight percent of the adduct.

The main drawback to this method is that when F_6 is extremely small, its proportion in the mother liquor cannot be calculated accurately. An alternative procedure is the

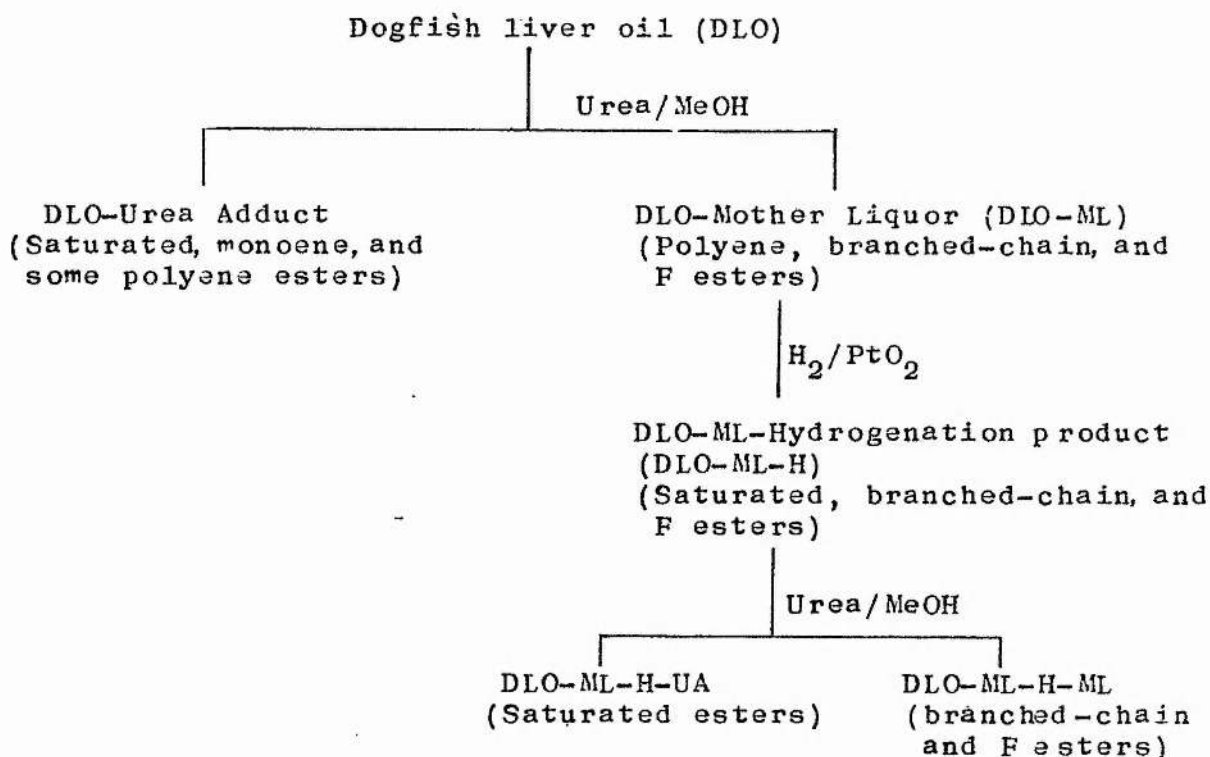
use of an internal standard. Such a standard must behave identically with the natural furanoid esters during the isolation process, but be distinguishable on GLC. An obvious choice is a synthetic furan. Although a variety of methods of preparing 2,5-disubstitued furans are now available (see Section II), these compounds are not suitable because they are not totally resistant to complex formation with urea. Scrimgeour¹⁵⁸ found that squalane is a suitable standard for this purpose. Ackman¹⁵⁹ has evaluated the nonyl and decyl esters of 3-cyclohexylpropionic acid as satisfactory internal standards for quantitative determination of isoprenoid acids after urea fractionation. The suitability of these for the quantitation of furanoid esters will depend on whether they behave identically with the natural material.

(v) Furanoid and Unusual Methyl-branched Acids in Dogfish Liver Oil

Methyl esters prepared from dogfish liver oil were crystallized with 5 times their weight of urea and the esters retained in the mother liquor were examined in detail.

When the mother liquor was hydrogenated using Adams catalyst and the product similarly crystallized with urea the furanoid esters and methyl-branched esters concentrated in the final liquor (Table 8).

Scheme 2 Isolation of Furanoid Esters From Dogfish Liver Oil by Procedures Involving Hydrogenation and Urea Crystallization



Comparison of the composition of this extract (DLO-ML-H-ML, Table 8) with that of the furan band isolated from the same oil by urea crystallization followed by Ag^+ TLC (Table 12) shows that the latter contains more F esters relative to the three phytol-based esters. This may be due to destruction of some F esters by hydrogenation. Although selective hydrogenation of normal unsaturated esters in the presence of F esters is possible by use of Adams catalyst, this process requires extreme care and often leads to partial hydrogenation of the F esters.

In a large-scale experiment, methyl esters from dogfish liver oil were crystallized with excess of urea

Table 8: Isolation of furanoid esters from dogfish liver oil by procedures involving hydrogenation and urea crystallization

<u>Identification</u>	<u>DLO-ML-H</u> [*]	<u>DLO-ML-H-UA</u>	<u>DLO-ML-H-ML</u>
14:0	-	0.1	-
15:0	0.4	0.2	1.0
16:0	3.0	4.3	-
17:0	-	0.1	-
18:0	9.3	15.8	-
19:0	0.4	0.5	0.7?
20:0	20.3	36.2	0.5
21:0	1.3	2.1	0.5?
22:0	49.8	35.7	-
23:0	-	-	-
24:0	-	0.3	-
F ₁	0.8	-	2.4
F ₂	1.0	-	5.8
F ₃	- ^a	-	1.8
F ₄	2.7	-	6.3
F ₅	0.5	-	2.8
F ₆	1.9	-	18.5
F ₇	-	-	0.7
P ₁₆	1.6 ^b	-	9.2
P ₁₉	1.5	-	9.5
P ₂₀	3.7	-	25.5
Other	1.8	4.7	14.8

* See Scheme 2

^a may overlap with 22:0

^b may also contain some 14:0

and the mother liquor fractionated by Ag^+ column chromatography. The bulk of the F esters and the P esters were contained in four fractions which eluted with PE5 (Fractions 2-5) and their compositions (by GLC) are shown in Table 9.

Fraction 1 did not show any GLC peaks and presumably contained only hydrocarbon^{carbons}~~generations~~. Fraction 2 contained mainly the three P esters (P_{16} , P_{19} , and P_{20}). Fraction 3 was a mixture of these three and F esters (F_1 - F_7) while the next fraction was composed of 78% of the latter. Two novel esters of ECL 18.7 and 20.7 accounted for 54% of fraction 5 and these two are referred to as U_1 and U_2 respectively.

Comparison of the distribution of individual F esters between the fractions 3 and 4 suggests that this fractionation is due to both difference in chain length as well as degree of ring-methylation. The longer-chain members such as F_6 seem to elute quicker than shorter-chain ones such as F_1 but the delayed elution of F_5 (note higher proportion of F_5 and also F_2 in fraction 4) would suggest that the mono-methylated F esters are held more strongly on the column than the di-methylated members. As a matter of fact, the synthetic non-methylated F esters such as 9,12- and 10,13-furans can be satisfactorily separated from the natural methylated F esters by silicic acid TLC.

The characterization of each fraction was based mainly on GC-MS and was supported by PMR and IR data. For example, fraction 2 showed a group of five PMR signals at

Table 9: Isolation of furanoid esters from dogfish liver oil by urea crystallization followed by Ag^+ column chromatography of the liquor

<u>Identification</u>	<u>Composition (weight %) by GLC</u>				
	Fraction:	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>
P ₁₆		18	10	3	3
P ₁₉		20	4	-	-
P ₂₀		56	15	3	3
F ₁		-	2	5	3
F ₂		-	5	15	12
F ₃		-	3	5	3
F ₄		-	12	17	3
F ₅		1	5	9	2
F ₆		5	42	26	6
F ₇		-	2	1	-
U ₁		-	t	5	47
U ₂		-	-	3	7
Other*		-	-	8	11

* May include diene and triene esters of C₁₆ and C₁₈ acids

0.90-1.02 indicative of the presence of methyl branched-chain structures characteristic of phytol-based esters. These signals were also present in the PMR spectrum of fraction 3, which in addition contained ^{na}signlets at 1.78 and 1.84 (methyl substituents on the furan ring) and a triplet at 2.42 (methylene groups α to the ring). The PMR spectrum of fraction 4 contained all the signals expected of ring-methylated F esters but that of fraction 5 contained none of these signals. Furanoid esters, methylated as well as non-methylated, show characteristic IR absorptions and while the spectra of fractions 3 and 4 contained these signals, they were absent from that of fraction 5 [see Experimental Section I.7 (d) for full spectroscopic data and Section III for their interpretation].

Hydrogenation using Adams catalyst caused little change in fraction 3 or 4 but the two unusual components U_1 and U_2 in fraction 5 were each converted to double peaks of ECL 16.3 and 18.3 (Table 10). Non-methylated F esters undergo hydrogenation more easily than methylated ones but it seemed unlikely that the mild conditions used were sufficient to effect their complete hydrogenation. Besides such reaction would have led to tetrahydrofuran esters whose ECL's do not usually differ greatly from that of the original F esters.

Table 10: Catalytic Hydrogenation of Fraction 5

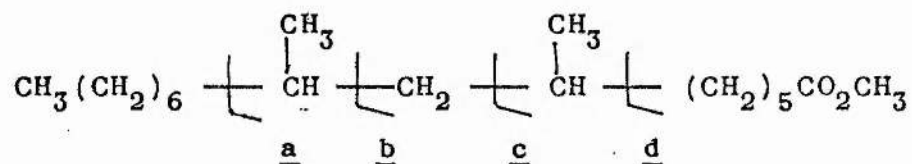
<u>Identification</u>	<u>Fraction 5</u>	<u>Fraction 5-H</u>
P ₁₆	14.1(3)	14.1(2)
P ₁₉	-	-
P ₂₀	17.0(3)	17.0(5)
F ₁	20.3(3)	20.3(2)
F ₂	21.2(12)	21.2(14)
F ₃	21.8(3)	21.8(1)
F ₄	22.2(3)	22.2(3)
F ₅	23.1(2)	23.1(2)
F ₆	23.7(6)	23.7(6)
U ₁	18.7(47)	-
U ₂	20.7(7)	-
U ₁ -H*	-	16.3(45)
U ₂ -H*	-	18.3(10)
Other	(11)	(10)

* These refer to products arising from U₁ and U₂ after hydrogenation

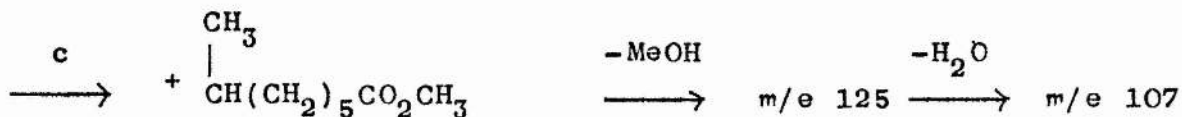
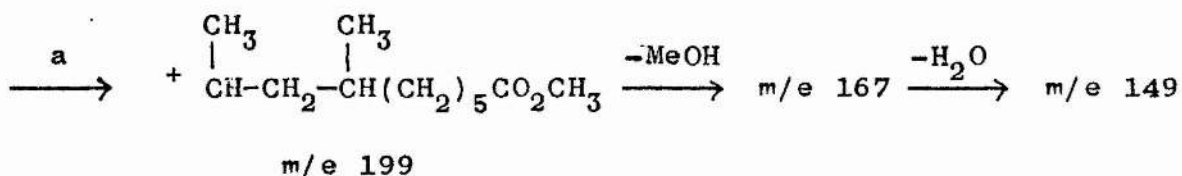
Co-injection of authentic methyl palmitate and methyl stearate with the hydrogenation product of fraction 5 gave additional peaks around ECL 16.3 and 18.3 showing that the two unusual peaks were not due to them.

Compound U₁

GC-MS of fraction 5-H showed that the compound of ECL 16.3 (U₁-H) had a molecular weight of 298. Although C₁₇ tetrahydrofuran or tetrahydropyran structures are possible for this molecular weight, no fragment ions in support of such compounds were present in the mass spectrum. A dimethyl-branched C₁₆-chain structure was consistent with the ECL of 16:3 and indeed the mass spectrum provided strong evidence for such a compound.



Fragment ions at m/e 199(a) and 171(b), and also at 157(c) and 129(d), showed the presence of methyl branches on C-7 and C-9 respectively. The structural assignments for the fragment ions at m/e 199 and 157 were confirmed by sequential loss of 32 and 18 mass units from each of these ions, which are due to elimination of methanol followed by water¹⁶⁰.

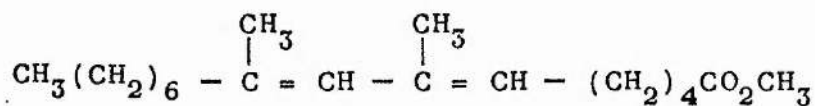


Additional support is provided by the presence of the characteristic ions produced by rearrangement of one or two hydrogens to the α -cleavage ions (b) and (d) (172, 173 and 130, 131 respectively).

The mass spectra of each component of the double peak of ECL 16.3 were identical. Thus its GLC separation must be due to different diastereoisomers.

GC-MS of fraction 5 showed that the molecular weight of U_1 is 294 which indicates that it contains two units of unsaturation. Because of its low ECL it is unlikely that U_1 contains a hetero atom. The dimethyl-branched acid (U_1 -H) could have been produced by the hydrogenation of a C_{18} dimethylene acid but the ECL of U_1 (18.7) is too low to account for such a compound. Therefore U_1 is a dimethyl-branched acetylenic or a dienoic acid. A decrement of 2.4 ECL units upon hydrogenation is compatible with an acetylenic structure but the CMR spectrum of fraction 5 did not show any evidence for triple bonds. Hence U_1 is most probably a diene and the ECL suggests that the double bonds are in conjugation.

Such a conjugated diene structure was supported by strong UV absorption at 228 nm. The PMR spectrum of fraction 5 contained a finely split doublet at 1.66

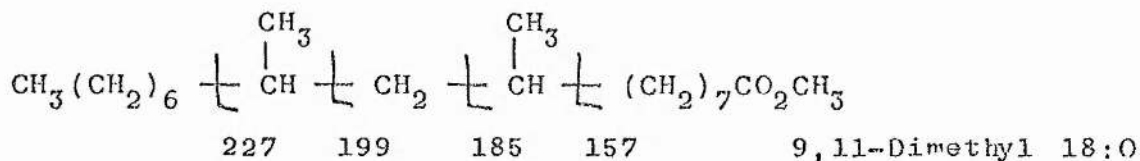
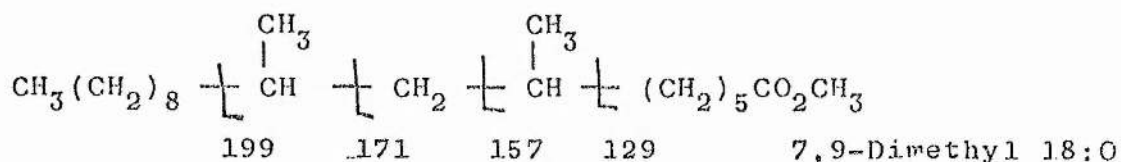


which is evidence for vinylic methyl groups. The small coupling ($J=5Hz$) between the methyl and vinyl hydrogen

suggests cis-geometry for the double bonds. A multiplet representing the vinyl protons was present at ~ 5.5 .

Compound U₂

The mass spectrum of the perhydro derivative of U₂ obtained by GC-MS of the hydrogenation product of fraction 5, contained a molecular ion peak at m/e 326. Thus it seemed likely that U₂ is a higher (C₂₀) homologue of U₁.



Its mass spectrum displayed ions at m/e 199 and 157, and ions corresponding to sequential loss of 32 and 18 mass units from each of them. Presence of ions at m/e 171 and 129 accompanied by hydrogen rearrangement ions (172, 173 and 130, 131 respectively) suggested close structural similarity to the perhydro derivative of U₁ (7,9-dimethyl substitution). Additional ions at m/e 227 and m/e 185 together with ions corresponding to sequential loss of methanol and water $\left[227 \xrightarrow{-32} 195 \xrightarrow{-18} 177; \text{ and } 185 \longrightarrow 153 \longrightarrow 135 \right]$ arise from a 9,11-dimethyl substituted ester. This was confirmed by peaks at 199 and 157 with the corresponding hydrogen rearrangement ions (200, 201 and 158, 159 respectively).

Hence U_2 is a mixture of di-unsaturated derivatives of 7,9- and 9,11-dimethyl 18:0. The double bonds are probably conjugated but their positions are not certain.

The component acids of 22 lipid samples from 15 different species were examined in the present study. Details of these samples are set out in Table 11.

(vi) Composition of the Furan Band

The furan band obtained by Ag^+ chromatography of the filtrate remaining after urea fractionation of the total methyl esters was usually composed of three branched-chain esters based on phytol (P_{16} , P_{19} and P_{20} : range, 9-87%; mean, 32%), up to nine furanoid esters (F_1 - F_9 : range, 0-76%; mean, 41%), and two unusual components designated U_1 and U_2 (range, 0-29%; mean, 4%) [Table 12]. The occurrence and distribution of furanoid esters in fish lipids are discussed in the following section.

The occurrence of P_{16} , P_{19} , and P_{20} in fish lipids is well documented^{72,95,104,77,106} and the present work confirms their wide distribution in these lipids. Among them phytanic acid, P_{20} (range, 2-26%; mean 13%) is the most common followed by P_{16} (range, 3-31%; mean 11%), though the least abundant pristanic acid (P_{19} : range, 1-36%; mean 7%) was dominant in the body lipids of the haddock and herring. As previously noted by Ackman⁷⁷ there seems to be no significant pattern of occurrence of these isoprenoid acids between freshwater and marine sources.

The two unusual components U_1 and U_2 occur only

- a Loch Lomond (Scotland),
- b West Coast of Scotland,
- c North Sea (off Aberdeen),
- d Antarctic,
- e Gatty Marine Laboratory, St. Andrews,
- f Commercially refined oils (Marfleet refining company, Hull),
- g Torry Research Station (Aberdeen).

Table 11: Source of Experimental Samples

Common name	Zoological name	Geographical source and date of capture	Sex	Tissue extracted	Lipid content	Spawning period
<u>freshwater samples</u>						
Roach	<u>Leuciscus rutilus</u>	a 10/75 a 10/76	M F	liver	M, 1.0; F, 2.5	June
Powan	<u>Coregonus lavaretus</u>	a 10/75	M, F	liver	M, 6.6; F, 5.3	Jan.
Brown Trout	<u>Salmo trutta</u>	a 10/75	F	liver	3.6	Dec-Jan
Perch	<u>Perca fluviatilis</u>	a 10/75	F	liver	3.1	May
Pike	<u>Esox lucius</u>	a 8/76	M	liver	10.7	May
<u>freshwater/marine samples</u>						
Sea Trout	<u>Salmo trutta</u>	a 10/75	M, F	liver	M, 2.5; F, 2.7	Dec-Jan
Salmon	<u>Salmo salar</u>	b 8/75	unknown	liver	10.0	Dec-Jan
<u>marine samples</u>						
Haddock	<u>Melanogrammus aeglefinus</u>	c 5/77	unknown	flesh	0.7	March
Herring	<u>Clupea harengus</u>	c 5/77	unknown	flesh	12.9	Feb-March
Mackerel	<u>Scomber scombrus</u>	c 5/77	unknown	flesh	10.3	
Ice fish	<u>Chaenocephalus aceratus</u>	d 6/76	M	liver	9.2	
Octopus	<u>Eledone cirrhosa</u>	e 4/76	M	digland	15.4	
Dogfish	<u>Squalus acanthias</u>	f -	mixed	liver	-	
Capelin	<u>Mallotus villogus</u>	f -	mixed	body	-	
Cod	<u>Gadus morhua</u>	f -	mixed	liver	-	March
Cod	"	g -	mixed	liver	-	March
Cod (fed)	"	g 5/77	F	liver	56.2	March
Cod (starved)	"	g 5/77	1M+1F	liver	4.8	March
Cod (starved)	"	g 4/76	F	liver	4.6	March

Table 12: Component Acids (% wt) of Furan Bands taken from Ag^a

species sex	ECL ^a	roach (m)	powan (m)	pike (m)	roach total CE ^b (f)	powan (f)	salmon total CE ^c	ice fish (m)	octopus (m)	haddock ?	herring ?	mackerel ?	dog (m)
total (%)		5	2	19	10	3	3	6	1	1	1	1	
P acids		(14)	(31)	(17)	(9)	(12)	(25)	(32)	(27)	(64)	(87)	(28)	(2)
P ₁₆	14.1	11	8	7	4	3	13	5	14	6	31	14	1
P ₁₉	15.8	1	11	1	2	5	1	1	3	36	33	1	
P ₂₀	17.1	2	12	9	3	4	11	26	10	22	23	13	1
F acids		(76)	(46)	(71)	(51)	(67)	(68)	(64)	(0)	(0)	(0)	(22)	(6)
1	20.5	-	2	-	1	4	2	3	-	-	-	-	
2	21.4	1	16	-	Tr	4	3	4	-	-	-	9	1
3	22.0	1	2	-	-	4	Tr	5	-	-	-	1	
4	22.4	12	7	-	8	14	14	33	-	-	-	5	1
5	23.3	2	6	6	1	3	10	1	-	-	-	4	
6	23.9	59	10	6	41	38	39	18	-	-	-	3	2
7	24.3	1	3	1	-	-	Tr	-	-	-	-	-	
other	-	-	-	58	-	-	-	-	-	-	-	-	
other acids													
U ₁	18.9	-	2	-	-	-	5	1	-	-	6	29	
U ₂	20.9	-	2	-	-	-	Tr	-	-	-	2	1	
others		10	19	12	40	21	2	3	73	36	5	20	

^a Values from DEOS column^b These refer to the total esters present as cholesterol esters and to the esters from this source travelling in the furan band^c This furan band is obtained in the usual way from the esters present in the cholesterol ester fraction

in exceedingly small quantities. They appear to be more common in lipids from marine fish than in those derived from freshwater sources.

(vii) Distribution of Furanoid Fatty Acids in Fish Lipids

Furanoid acids are widely distributed in fish lipids at a low level (Table 13). They were detected in the liver lipids of roach (male and female), powan (male and female), pike (male), ice fish (male), salmon, dogfish, and cod and in the body lipids of a mackerel. F acids were absent (or present at a level below 0.2%) from the liver lipids of sea trout (male and female), brown trout (male and female), perch (female), and from the body oils of herring, haddock and capelin. They were also absent from the digland of the octopus and from several fish meal samples¹⁶¹.

Glass and coworkers showed that, in general, there are greater concentrations of F acids in the testes than in the liver of most fish¹⁶². We did not examine testicular lipids but we analysed several body lipids. Only in mackerel were F acids found in a body lipid.

F acids have been detected in blood serum lipids of cod¹⁶³, pike¹⁶², and brook trout¹⁶². Complete removal of blood from samples of flesh is not easy and it is possible that the small amounts of F acids found in the mackerel flesh were due to such contamination.

Glass and coworkers observed high levels of F acids in the liver of male pike^{11,12} and more recently

Table 13: Content (% wt) of Furanoid Acids in the Lipids of Freshwater and Marine Fish

<u>Freshwater</u>	<u>Tissue</u>	<u>Male</u>	<u>Female</u>	<u>Mixed</u>	<u>Unknown</u>
Roach	L	4	5		
Powan	L	1	2		
Pike	L	13			
Brown trout	L	-	-		
Perch	L		-		
<u>Freshwater/Marine</u>					
Salmon	L				2
Sea trout	L	-	-		
<u>Marine</u>					
Ice fish	L	4			
Dogfish	L			1	
Cod	L		0.5	1	
Capelin	B			-	
Mackerel	B				0.2
Herring	B				-
Haddock	B				-
Octopus	D	-			

L = liver, B = body, D = digland

- indicates less than 0.2%

reported similar concentrations (up to 33%) in several spawning male freshwater fish¹⁶². Such abnormal levels were not found in the lipids of any of the healthy fish examined by us though it is of interest that the highest level of F acids we encountered was also in the liver of a male pike (13%). The composition of F acids in this sample, however, was unusual in that it contained more of the longer-chain members (F₈ and F₉) and more of the unsaturated derivatives than any of the other samples. F₁-F₅ were absent altogether from this pike liver.

We found furanoid acids in freshwater as well as in marine fish (Table 13). Salmon and sea trout live in freshwater and marine environments and whilst the salmon contained F acids the trouts did not. The ice fish live in the unique antarctic conditions and the presence of F acids in this species is particularly noteworthy.

The freshwater fish (range, 1-13%; mean, 5%) contained slightly more F acids than the marine fish (range, 0.5-4%; mean, 1.4%) but the number of marine fish lipids examined is not sufficient to draw any conclusions. Scrimgeour has reported these acids in both crude and refined cod liver oils around the 1% level¹⁵⁸.

Glass et al.¹⁶² related the high content of F acids in their samples of pike and other freshwater fish to their male sex but we found these acids in female as well as in male fish. We did not find any significant difference in occurrence or in amount due to difference in sex (Table 13). Within the limited range of samples we

were able to study, F acids were either present in (roach, powan) or absent from (brown trout, sea trout) the lipids of the male and female.

Nor is there any significant pattern of occurrence of F acids with the zoological family. Among the four Salmonidae examined, salmon and powan contained F acids whilst the sea trout and the brown trout did not. Presence of F acids in powan but not in trout is interesting because they are closely related species.

Occurrence of F acids have not been reported thus far in animals other than fish. They were absent from the digland of the octopus examined by us but recent work in this laboratory has shown the presence of small quantities of these acids in the mammalian porpoise¹⁶⁴.

Table 14 shows the relative composition of the individual F acids in the furanoid extracts. F_6 is almost always the dominant member of the series comprising 38-80% (mean, 57%) of the total F acids. Exceptions were the powan (male) and mackerel where F_2 was the major component. After F_6 , the next most common member is F_4 (range, 9-23%; mean, 16%) followed by F_2 (range, 1-19%; mean, 10%) and F_5 (range, 2-15%; mean, 9%). F_1 (range, 0-14%; mean, 4%) and F_3 (range, 0-8%; mean, 3%) are relatively minor components and may even be absent altogether. F_7 (range, 1-6%; mean, 1%) was often exceedingly small and this component has been ignored in some analyses.

Table 14: Relative Composition of Individual F Acids in Furanoid Extracts
from Fish

Acid	Sex*	Roach		Powan		Pike	Salmon	Ice fish		Mackerel	Dogfish		Cod	
		m	f	m	f	m	?	m		?	mixed	f	mixed	mixed
F ₁		-	2	4	6	-	3	4		-	3	9	1	14
F ₂		1	-	35	6	-	4	6		40	17	19	13	14
F ₃		1	-	4	6	-	-	8		5	5	2	4	1
F ₄		16	16	15	21	-	21	52		23	17	9	15	10
F ₅		3	2	13	4	8	15	2		18	8	13	11	12
F ₆		78	80	23	57	8	57	28		14	48	46	55	38
F ₇		1	-	6	-	2	-	-		-	2	2	1	1
Other		-	-	-	-	82	-	-		-	-	-	-	10
Σ (n-4) acids		17	18	25	27	2	24	56		23	22	20	17	25
Σ (n-6) acids		83	82	75	73	16	76	44		77	78	80	83	65
Σ Mono-methyl acids		96	98	52	90	92	81	92		42	75	68	76	74
Σ Di-methyl acids		4	2	48	10	8	19	8		58	25	32	24	26

* m = male, f = female

The furanoid acids with n-6 structure (range, 44-83%; mean, 75%) usually predominate over those with n-4 structure (range, 17-56%; mean, 25%) and those with two methyl substituents on the ring (range, 42-98%; mean, 78%) are more common than those with only one (range, 2-58%; mean, 22%). Ice fish is unique in having more n-4 than n-6 acids due mainly to the high content of F₄. Powan (male) and mackerel differ from the others in containing larger proportions of the mono-methyl acids.

(viii) Distribution of Furanoid Acids in the Different Lipid Classes

We agree with the observation made by Glass et.al.^{12,162} that F acids in the liver lipids are usually concentrated in the cholesterol esters, are also present in the triacylglycerols, and appear to be absent in the phospholipids. For example, roach (female) lipid (see Table 20) showed a small peak in its total ester chromatogram which may be the most prominent of the F acids (F₆, 2.9%) but this same peak accounted for 36% of the cholesterol esters. Such concentrations of F acids in the CE fractions, though less marked, were also observed in the lipids of the salmon and the cod (fed).

We have also noted an unequal distribution of F acids in the different lipid classes. F₄ and F₆ seem to be preferentially esterified to cholesterol. F₂, on the other hand is present more often in the triacylglycerols. In the liver lipids of the salmon and roach, F₂ was almost completely absent in the CE fraction (Table 15).

Table 15: Distribution of the F Acids in the Furan Bands from Component Lipids of some Fish Livers

	<u>Roach(f)</u>		<u>Salmon</u>		<u>Cod(fed)</u>			
	<u>Total</u>	<u>CE</u>	<u>Total</u>	<u>CE</u>	<u>Total</u>	<u>CE</u>	<u>TG</u>	<u>PL</u>
F ₁	0.8	-	1.8	-	3.7	-	2.3	-
F ₂	0.3	-	2.6	0.2	8.6	-	8.9	-
F ₃	-	-	0.3	-	1.3	?	1.0	-
F ₄	7.8	12.3	14.0	14.4	4.1	?	1.3	12.2
F ₅	1.0	2.2	10.4	13.5	5.9	?	1.7	6.6
F ₆	41.3	72.7	38.5	45.1	22.0	8.2	0.7	12.3
Other	48.8	12.8	32.4	26.8	54.4	?	84.1	68.9

It was dominant^a in the TG's of the cod (fed) liver lipids and was absent from the CE and PL fractions.

(ix) Effect of Starvation

Fish withstand astonishingly long periods of total abstention from food. Love⁶⁵ found that cod (Gadus morhua) can survive more than 195 days without food. Herring (Clupea harengus)¹⁶⁵ survived for 129 days and Amica calva¹⁶⁶ managed to survive for 20 months without food. Anguilla anguilla¹⁶⁷ started to die only after they had been starved for 3 years and the lung fish (Protopterus), which slows down its metabolic rate to a low level during aestivation, when it is buried in the mud of dried-up ponds, lived in this state for even longer - three and a half years, according to the work of Smith¹⁶⁸.

We compared liver lipids^{of} cod starved for 4-5 months with those obtained from cod feeding normally, and found striking differences in their lipid composition and constituent fatty acids. (Table 16). The liver lipids of the fed cod contained the normal spectrum of fatty acids with the n-3 polyenes 20:5 and 22:6 and the monoene 18:1 dominant. The fatty acids of the liver lipids of starved cod on the other hand contained the furanoid acid F₆ as the major component (37 and 27% respectively). All other normal acids but 22:6(n-3) were relatively minor.

The livers of the starved cod yielded much less lipid than those of fed fish (Table 11). Those from the fed fish were mainly triacylglycerols (80%) with very little cholesterol esters (1%) whereas the lipids from the starved fish were mainly cholesterol esters (57%), the triacylglycerols having fallen to 6%.

The fatty acid composition of the phospholipids in the fed and starved fish are remarkably similar. Those of the triacylglycerols are also not very different but the cholesterol esters differ greatly in their composition with the F acids in particular concentrating in this lipid of starved fish at a level of 84%. (Table 16).

Table 16: Fatty Acid Profiles of the Total and Component Liver Lipids of Fed and Starved Cod

	Cod (fed) ^a			Cod (starved) ^b			Cod (starved) ^c		
	Total	CE (1%)	TG (80%)	PL (15%)	Total	CE (57%)		TG (6%)	PL (37%)
Saturated	(13.1)	(9.8)	(12.3)	(14.4)	(6.4)	(1.8)	(10.3)	(13.2)	(20.4)
14:0	1.5	1.6	1.8	2.0	1.4	1.3	3.1	1.6	11.1 ^d
16:0	8.9	5.8	8.7	9.9	4.0	0.5	6.5	8.2	7.7
18:0	2.7	2.4	1.8	2.5	1.0	-	0.7	3.4	1.6
Monoenes	(35.2)	(33.1)	(32.6)	(24.5)	(11.3)	(5.3)	(46.8)	(21.6)	(13.2)
16:1	5.9	6.9	6.5	4.5	0.7	0.2	2.7	1.2	1.0
18:1	19.4	20.7	17.6	13.9	4.7	2.0	7.7	9.4	7.7
20:1	4.9	5.5	4.7	3.7	1.6	1.2	8.0	3.1	1.9
22:1	3.9	-	3.0	2.4	4.3	1.9	25.1	7.9	2.6
24:1	1.1	-	0.8	-	-	-	3.3	-	-
n-6 Polyenes	(6.2)	(11.3)	(6.2)	(8.6)	(2.4)	(0.9)	(6.2)	(9.8)	(3.0)
18:2	1.9	2.6	2.2	1.5	0.8	0.4	1.5	1.4	0.4
18:3	-	5.6	-	-	0.3	0.3	1.4	1.5	-
20:3	0.3	1.5	0.1	-	-	-	0.9	0.8	-
20:4	2.3	1.6	2.1	4.9	0.5	0.2	0.5	3.3	2.3
22:4	0.8	-	1.1	1.4	-	-	1.7	1.0	-
22:5	0.9	-	0.7	0.8	0.8	-	0.2	1.8	0.3
n-3 Polyenes	(41.6)	(34.5)	(45.5)	(51.1)	(30.6)	(6.9)	(32.4)	(50.6)	(28.0)
18:3	-	3.8	-	-	-	-	-	-	0.1
18:4	1.3	3.8	1.8	1.7	0.3	-	1.0	0.5	-
20:4	0.6	2.4	1.0	0.6	-	-	-	-	-
20:5	15.2	14.9 ^e	16.2	12.9	3.2	-	3.9	10.4	5.3
22:5	2.6	?	2.8	2.8	1.2	0.8	4.5	3.5	1.7
22:6	21.9	9.6	23.7	33.1	25.9	6.1	23.0	36.2	20.9
F ₆	0.4	8.2 ^f	0.2	-	36.6	67.0	-	1.1	26.6
Other	3.5	3.1	3.2	1.4	12.7 ^g	18.1 ^g	4.3	3.7	8.8 ^g

^a Single female; ^b One male and one female; ^c Single female; ^d This unusually high proportion of 14:0 probably represents contamination with antioxidant; ^e may contain F₄; ^f may contain 22:5(n-6); ^g contain other F esters.

Again we observe an unequal distribution of the several F acids in the different lipid classes (Table 17). The cholesterol esters contain mainly F₄, F₅, and F₆ and the lower members F₁-F₃ are virtually absent from this lipid class. The triacylglycerols, on the other hand, has F₂ as the major F acid. Like the cholesterol esters, the phospholipids contain F₄-F₆, but the proportion of F₆ in the latter is less than that in the former.

The total esters and the cholesterol esters of the lipids of the starved fish were each separated into two furan bands. The more polar band, which migrated like monoene esters, contained four late-eluting GLC peaks as major components. They comprised 1-2% of the CE fraction and were probably monounsaturated F esters.

Phytol-based esters were present in the lipids of both fed and starved cod but there was no significant distribution pattern between the different lipid kinds. (Table 17).

Glass et.al. related the high content of furanoid acids in their samples of pike¹² and other freshwater fish¹⁶² to their male sex, but in a preliminary study of pike lipids carried out in this laboratory¹⁶⁹, little difference was found between the F acid content of the ovary and the testes (both around 10%). As already pointed out, the F acids occur in the liver of both male and female fish, with no significant difference in amounts. We consider that the nutritional status of the animal is more significant with the F acid levels rising in both male

Table 17: Composition of the Furan Bands of the Total and Component Lipids
from the Livers of Fed and Starved Cod

	Cod (fed)						Cod (starved)				
	Refined		Crude		Single Female		Single Female		1 Male + 1 Female		
	Total	Total	Total	TG	PL	Total	Total(A) ^a 40-50	Total(B) ^a	CE(A)	CE(B)	PL
Furan band (%)	1	1	1	<1	<1	40-50		1	84	1-2	<1
Phytol-based acids											
P ₁₆	10	6	7	17	11	1	2	-	1	1	14
P ₁₉	Tr	5	Tr	-	-	1	Tr	-	-	Tr	3
P ₂₀	7	13	14	34	11	Tr	2	-	1	1	31
Furanoid acids											
F ₁	1	10 ^b	4	2	-	1	Tr	-	Tr	-	1
F ₂	10	10	9	9 ^b	-	-	1	2	Tr	-	- ⁶⁷
F ₃	3	1	1	1	-	2	2	1	1	-	-
F ₄	12	7	4	1	12	20	13	2	12	3	6
F ₅	9	9	6	2	7	2	4	3	5	1	2
F ₆	43	28	22	1	12	72	72	15	73	16	5
F ₇	1	1	1	-	-	-	-	-	-	-	-
Other	-	-	-	-	-	-	3	31 ^a	3	63 ^a	13
Other acids											
U ₁	1	4	3	18	-	-	-	2	-	-	-
U ₂	Tr	?	1	?	-	-	-	-	-	-	-
Others	3	6	28	15	47	1	1	44 ^a	4	15	25

^a Total esters and cholesterol esters each separated into two furan bands: the second was more unsaturated and contained 18:2 (23%) and probably unsaturated F acids.

^b may contain some U₂

and female starved fish.

Although some organs do not show measurable changes during starvation, even up to the point of death, the liver is affected soon after the cessation of feeding. For example, cod brain (Gadus morhua), with 8.8% lipid shows no reduction of this figure even when the fish are dying of starvation, although the liver lipids have fallen from about 40% right down to 2%.

Our two starved cod liver samples contained only 4.8 and 4.6% of lipid compared with 56% for a healthy fish showing that these two fish had undergone severe depletion of lipid reserves. The lipid content cannot be taken as a reliable index of nutritional status because as the fish grows it contains increasing amounts of lipid in the liver. However, g liver lipid/Kg fish is the same for a given length of the fish and the ratio of g liver lipid/Kg of starved fish to that of the fed fish of the same length is a measure of the nutritional status¹⁶³. The fed cod had a nutritional status of 85.8 and the two starved fish had values of 2.6 and 2.4 which represented extremely poor nutritional states.

Different lipid classes are not depleted at the same rate. Generally, the triacylglycerols can be drawn upon easily when food is scarce. In mammals the phospholipids form part of the structure of body tissues, for example, cell walls, and so cannot be utilized during starvation¹⁷⁰. However, starvation of Oncorhynchus tshawytscha¹⁷¹ and Gadus morhua (cod)¹⁶⁵ causes a fall

in PL as a proportion of the total lipid. Wilkins¹⁶⁵ showed that a reduction in the proportion of PL in Clupea harengus (herring) during starvation coincides with a reduction in certain protein fractions, indicating a breakdown in body tissues. It may be that the greater facility of fish for utilizing protein during starvation enables them to utilize PL at the same time, whereas in the higher vertebrates the same degree of cellular disorganization would be disastrous⁶⁵.

As already mentioned, the greater proportion of the F esters occur in the cholesterol esters. If the TG's and PL's are depleted in preference to or at a much faster rate than the CE's, then the relative enrichment of F acids in the starving cod may be regarded simply as due to a concentration effect. Consideration of the absolute amounts of different lipid classes in the fed and starved cod shows that the TG's and PL's are indeed utilized in preference to the CE's (Table 18). But, at the same time, the amount of CE's have increased five fold and it appears that some have been actually made during starvation.

Table 18: Changes in the Amounts* of Different Lipid Classes During Starvation of Cod.

	<u>Fed</u>	<u>Starved</u>
CE	0.56	2.7
TG	44.8	1.7
PL	8.4	0.3

* Expressed in g per 100 g of liver

Glass and coworkers observed wide seasonal fluctuations of the amounts of F acids in the liver and testes lipids of the northern pike¹² and several other freshwater fish¹⁶². For example, in brook trout and northern pike the levels of F acids reach a maximum in the testes lipids, and a minimum in the liver lipids, at spawning. When spawning approaches, the testes of these fish enlarge and become fatty. Concurrent with these changes the livers, which prior to that have contained (example, brook trout) 15-20% lipid whose fatty acids includes 10-15% F acids, decrease markedly in both. At spawning the livers contain 4-6% lipid and 0-5% F acids. Since the decrease in F acids in the liver coincides with their accumulation in the testes, Glass et.al. concluded that they have been transported from one to the other.

This hypothesis was supported by the presence of F acids in the blood lipids during this period.

The production of eggs or sperm always depletes a fish⁶⁵, and this condition of depletion associated with spawning is usually accompanied by a lack of desire to feed. For example, salmon ascending the rivers to spawn, abstain from food throughout the journey and, as a result, suffer an incredible amount of depletion, losing up to 99% of their lipid¹⁷². Assuming that our results with starved cod are generally applicable to other fish, then one might expect a relative increase in the level of F acids in the liver lipids of spawning fish. Paradoxically, the findings of Glass et.al.¹⁶² show that the decreases in liver lipids

in spawning fish is accompanied by a decrease in F acid levels.

It is not always possible to differentiate clearly the effects of starvation from those of maturity and spawning. But from the work of Glass et.al.¹⁶² it is clear that during the spawning period of a fish, its liver loses weight while the gonads gain weight. Starvation, on the other hand, causes loss in weight of both organs⁶⁵.

One way of separating the two causative factors of starvation is by castration. Ross¹⁶³, investigating the change in F acid levels in starving cod, found no difference between castrated males or females and normal fish. Therefore, at least as far as F acids are concerned, the depletive effects of maturation and spawning are different from the effects of merely withholding food at a time when the gonads are inactive.

Our results have established the indiscriminant occurrence of F acids in both male and female fish. Because of this, it would be interesting to know whether, at spawning a translocation of F acids from liver to gonads takes place in female fish too, similar to what has been observed by Glass et.al.¹⁶² in male fish. It is possible that the effect is more pronounced in the female because at spawning the female gonads take up a greater proportion of the body weight than the male gonads do⁶⁵.

The extremes of starvation described here for salmon are not suffered by cod, which feeds fairly intensively throughout the year¹⁷³. Seasonal variation of the muscle

and liver fatty acids of the cod has been investigated^{67,68} and although these studies have shown slight variations in the proportions of certain common fatty acids appreciable quantities of F acids have not been reported.

Despite previous studies^{165,174-176} on changes occurring during starvation, no reference has been made to unusually high concentrations of F acids and it would be of interest to see whether this happens in species other than cod. Kluytmans and Zandee¹⁷⁴ found only small differences in the fatty acid composition between the total body lipids of pike starved for two months and those fed twice a week. They, however, observed an unusual acid (23%) in the lipids of the testes, and considered this to be a 23:1 or 22:3 acid on the basis of the GLC behaviour, but did not identify it completely¹⁷⁵. It may be the major furan acid (F₆). Dave and coworkers¹⁷⁶ examining the metabolic and hematological effects of starvation in the european eel (Anguilla anguilla) observed the progressive elevation, during starvation, of an acid which they thought was 22:4. Like Kluytmans and Zandee, they did not identify it conclusively and it too could have been F₆.

We examined the liver and adipose lipids of rat but the lipids of other land animals have not been examined for F acids yet. The rat lipids did not contain any F acids and although starvation (24 h) caused significant changes in the level of 18:0, 18:1 and 18:2 no F acids could be demonstrated. A 24 h fast is perhaps not sufficient to

cause major changes and it would be interesting to know the effect of long-term fasting on the composition of liver lipid of rat and other land animals.

(x) Physiological Function of Furanoid Acids

Concentration of F acids in just two organs, namely, the liver and the testes; and their specific occurrence in CE (liver) and TG (testes) and absence from PL suggests a physiological function of these acids. In pike and brook trout the maximum concentration of F acids is attained at spawning time. Such observations led Glass et.al. to speculate that these acids perform some function during the fertilization process¹⁶². But in certain other fish examined by them, for example, bigmouth buffalo, though the liver lipids contained large amounts of F acids (as much as 40% of the total fatty acids), the testicular lipids contained very little of them even at spawning. Among their other samples, blugill, carpsucker, and rockbass all gave results similar to the bigmouth buffalo and only dogfish showed a build-up of F acids in the testes at spawning. It should be also pointed out that all of the fish we studied were captured outwith their respective spawning periods. According to the work of Glass et.al., this is the time when F acids are highest in the liver and yet, apart from the pike, the other samples did not contain unusually high concentrations of these acids. Such irregularities do not permit a precise definition of the

function of these acids.

Whatever their function, it is clear from our results that these F acids are not easily utilized by fish even during extreme starvation. Like brain lipids, which are not affected even up to the point of death, the F acids seem to survive until the latter stages of starvation. In the laboratory, cholesterol esters are more resistant to hydrolysis than other lipid kinds. If a similar situation exists in the living body it may explain at least in part, the persistence of CE and hence F acids during long spells of starvation. On the other hand, esterification to cholesterol could be a mechanism by which these acids, which may be expected to perform an important function are preserved in the body. The function of CE themselves is not clear³⁷.

Though the F acids, which occur mainly as CE in the liver are transported from that organ to the testes in the same form, they are deposited as triacylglycerols. Deposition, in this readily utilizable form may again suggest that these acids have been translocated to serve a specific function.

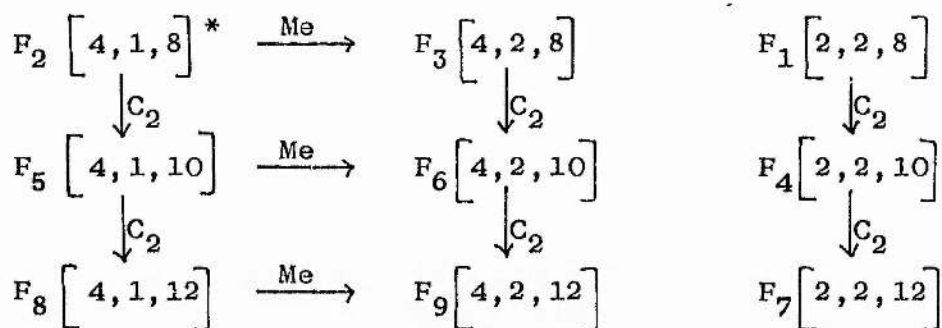
Presence of a five-membered ring in the middle of a fatty acid chain is reminiscent of the structure typical for prostaglandins. It is of interest in this connection that physiological activity resembling that of some prostaglandins have^s been claimed for several synthetic 2-furano- and 2-tetrahydrofurano-octanoates having a variety of substituents in position-5 of the ring.

All these compounds, however, contained an oxygenated function in the chain. Such a grouping may be necessary for these substances to exhibit prostaglandin-like activity, because Crundwell and Cripps¹⁷⁷ did not find such activity for their C₁₇ 9,12-furan acid. Nevertheless, it is possible that the presence of one or more ring-methyl groups is sufficient to induce prostaglandin-like activity to F acids. Testing of synthetic 9,12- and 10,13-furan as well as of natural F acids isolated from cod liver oil for such activity is underway*.

(xi) Biosynthesis

Biosynthesis of furanoid fatty acids is still a matter of conjecture. Of the furanoid acids so far discovered in fish lipids F₁, F₄, and F₇ have n-4 structures and the rest are n-6 acids. It is conceivable that they arise from 16:2(n-4) and 18:2(n-6) acids respectively, both of which occur in fish lipids. At the same time, it should be pointed out that the position of the ω double bond of a F acid is not necessarily the same as that in the precursor molecule, because the double bonds could rearrange depending on how the oxygen is introduced.

* Samples are being tested at "Wellcome" Laboratory, Kent.



* The first and last numbers in the parenthesis refer to the number of methylene groups separating the ring from the methyl- and carboxyl-ends respectively. The middle number indicates the number of ring-methyl substituents.

F_1 and F_2 may be considered as derived from 16:2(n-4) and 18:2(n-6) respectively. Chain-elongation of F_1 could then give F_4 and F_7 whilst F_2 produces F_5 and F_8 . The acids F_3 , F_6 , and F_9 may be envisaged as arising from methylation of F_2 , F_5 , and F_8 respectively.

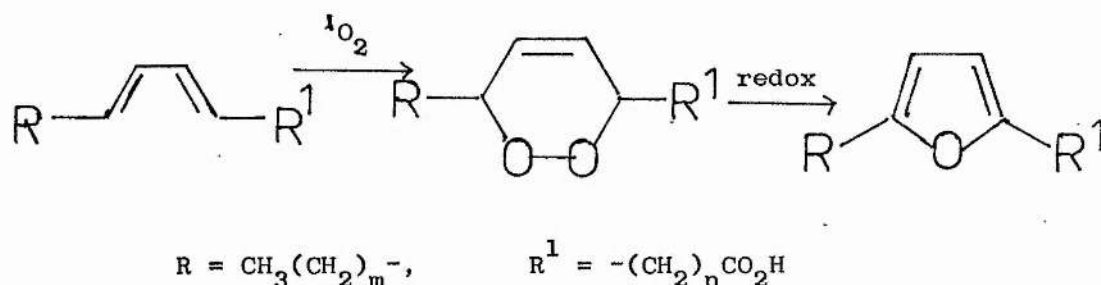
In an attempt to find out whether furanoid acids could be made by direct oxidation of unsaturated acids, we carried out a series of simple experiments. Neat samples of methyl linoleate, methyl linolenate, and methyl crepenynate (18:2 9c, 12a) respectively were exposed to a stream of oxygen over several days. After significant autoxidation had taken place, these samples were examined by the usual procedures of urea crystallization and Ag^+ TLC. Although the presence of hydroperoxides was observed we failed to detect either furanoid or cyclic peroxide esters in any of the samples.

Perhaps these oxidations would have been better carried out in the presence of a lipoxygenase. Two acids

containing a divinyl ether group have been described as products of linoleic and linolenic acid by action of Solanum tuberosum (potato tuber) lipoxygenase and sequential reactions^{178,179}. This is of interest because disregarding their cyclic structure, the furanoid acids also contain a divinyl ether group.

We have also shown [Section II (iii)] that furans are readily obtained by reduction of cyclic peroxides, which in turn, are produced from conjugated dienes by oxidation (Scheme 3). This sequence of reactions has

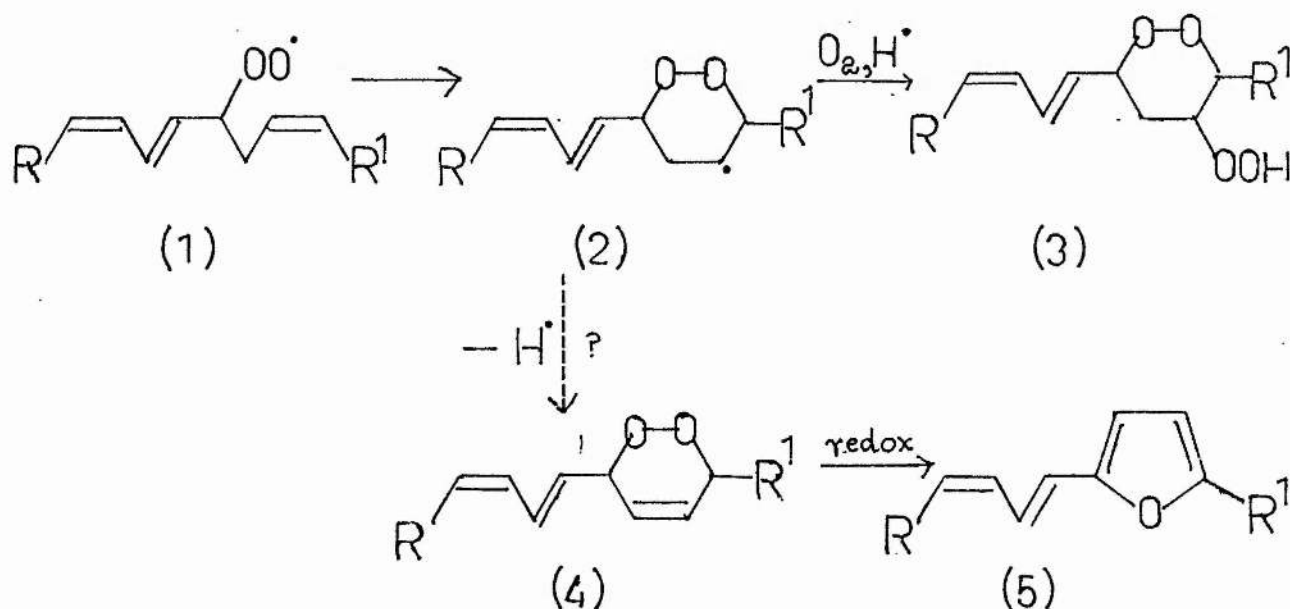
Scheme 3: Formation of Furans via Endoperoxides



now been widely accepted as the pathway by which furan^{an}o-terpenes are made in nature¹⁸⁰⁻¹⁸³, and has been substantiated by the circumstance that allylic alcohols coexist with conjugated dienes naturally (e.g. manool, the bioformenes, and solarene). The dienes, then, may be regarded as derived from the allylic alcohols. Conjugated dienes, however, have not been detected, so far, in fish lipids. Although a hydroxy oleic acid has been reported from castor oil fish¹⁸⁴, hydroxy acids are rare in fish lipids.

It is conceivable, though, that cyclic peroxides of the type shown in Scheme 3 are derived from normal

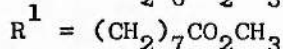
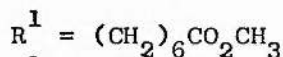
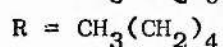
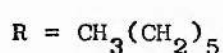
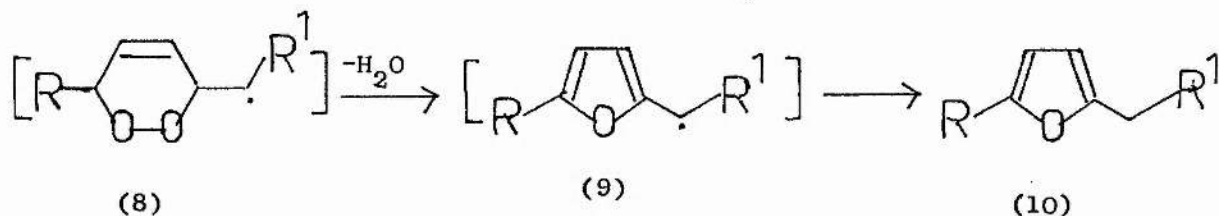
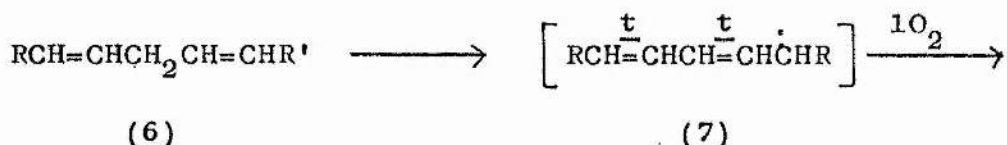
methylene-interrupted polyenes. It has long been known that autoxidation of linolenate gives more 9- and 16-hydroperoxides than the 12- and 13- isomers. Gunstone³⁴ suggested that the reduced yield of the latter may be due to their unique 1,5-diene structure leading to the formation of six-membered cyclic peroxide-hydroperoxides (3). Indeed, such products have been identified recently¹⁸⁵ and are considered to be formed via a six-membered cycloperoxy radical (2). A cyclic peroxide (4) may be produced from the intermediate (2) by elimination



of H^\bullet , instead of reaction with oxygen to form (3). Reduction of the peroxide (4) leads to the unsaturated derivative of 9,12-furan (5). It is noteworthy that the F acid isolated from Exocarpus cupressiformis seed is a 9,12-furan.

Autoxidation of linoleate furnishes two conjugated diene hydroperoxides 9-OOH 10_t,12_c and 13-OOH 9_c,11_t¹⁸⁶.

An alternative scheme set out below leads to 9,12- and 10,13-furans via 1,4-endoperoxides. The conversion of intermediate radical to neutral molecular^e is shown as the



last stage in the sequence but it could equally well occur with intermediates (7) and (8).

The occurrence of F acids substituted with one and with two methyl groups, but otherwise of very similar structure, suggests separate methylation steps in the course of their biosynthesis. However, a non-methylated F acid, which one might expect as an intermediate, has not been found yet in fish lipids. We have mass spectral evidence for the presence of trace amounts of non-methylated F acids. The possible existence of such compounds is complicated by the fact that these esters are not separated from their mono-methyl derivatives, on GLC. It should be also mentioned that such non-methylated F esters are not

completely resistant to adduct formation with urea - doing so with more or less the same facility as 18:2(n-6). Under the conditions of urea crystallization used by us and by Glass et.al. 18:2 is divided between the adduct and the mother liquor. If non-methylated F esters were present in any significant quantities, say 0.1%, they should have been detected in the liquor without much difficulty. If present only in trace amounts, however, the methodology used in the present studies would have precluded their detection.

It is unlikely that the F acids occurring in the lipids of fish originate in their diet. The freshwater fish examined in this study were taken from the same habitat and at the same time of the year, but the F acids were present in only some of them. Also the work of Glass et.al.¹⁶² have shown that in different fish the maximum levels of F acids are attained at different times, which is in conflict with an exogenous origin. In this connection, it is of interest that one of the F acids occurring in fish lipids, namely F₂, has recently been found in the lipids of rubber (Hevea brasiliensis) latex¹⁸⁷. This surprising discovery of F₂ in the lipids of a terre^strial plant substantiate the view that these compounds are derived from common precursors like linolenic acid.

(xii) Metabolism

Introduction

The discovery of furanoid acids in cod liver oil and in popular edible fish such as salmon¹⁶⁹ emphasized the need to ascertain whether dietary F acids are incorporated into animal tissue and what effects these acids may have on the animal. Besides furanoid acids, three kinds of cyclic acids have been recognised as minor constituents of edible oils: cyclopropane^{188,189} acids, cyclopropene¹⁸⁸ acids, and epoxy acids¹⁹⁰. Both cyclopropene¹⁹¹ and epoxy fatty acids have been held responsible for various physiological disorders in animals and these findings prompted us to look at the fate of F acids when fed to rats.

Results and Discussion

Two groups of rats were fed on diets supplemented with (a) 10,13-furan acid and (b) cod liver oil respectively. The fatty acid composition of the liver and adipose tissue of these rats were compared with that of a group of rats fed on the normal diet.

(a) Rats given 10,13-furan

Two groups of two female rats were fed on a commercially prepared diet* (12 g/day). The diet of one group was supplemented with 10,13-furan acid (50 mg/day). After 7 days the rats were killed and the livers and adipose tissue from each group pooled.

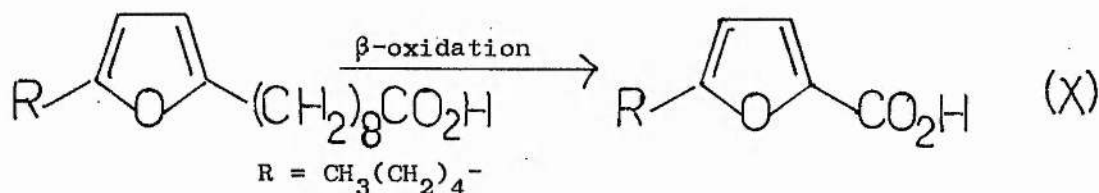
* 'Diet. 41B', supplied by North Eastern Agricultural Society, Banner Mills, Aberdeen. Its (rat cake) fatty acid composition is given in Table 20.

Rats in both groups lost weight during the seven-day period, presumably due to the reduced diet (normal diet is 15 g of rat cake a day). The loss in weight in the furan-fed group was only marginally lower than that of the control group (Table 19).

Table 19: Change in weight (g) of rats during furan-feeding

	CONTROL GROUP			FURAN-FED GROUP		
	Start	Finish	% loss in weight	Start	Finish	% loss in weight
Rat 1	280	271	3.2	326	302	7.3
Rat 2	356	325	8.7	334	303	9.2
Over-all loss in weight 6.2%						8.3%

In this relatively short-term experiment 10,13-furan acid in the diet was well tolerated by the rats. The fatty acid composition of the liver and adipose tissue of the furan-fed rats were in no way different from that of the standard groups and the presence of 10,13-furan or a metabolite of it were not observed. Nor were any furanoid compounds demonstrable in the feces of the furan-fed rats. Absence of furanoid-metabolites such as (x), which can be formed by β -oxidation of the 10,13-furan in the



diet is interesting because when cyclopropane fatty acids

Table 20: Fatty acid composition of tissues of rats fed on 10,13-furan acid and cod liver oil and that of the control group

	Liver (control)	Liver (10,13-furan-fed)	Adipose Tissue (control)	Adipose Tissue (10,13-furan-fed)
Saturated esters	(29.9)	(29.5)	24.2	(23.7)
14:0	0.1	0.1	1.1	1.4
16:0	13.4	12.7	17.5	17.6
18:0	16.4	16.7	5.6	4.7
Monoene esters	(8.9)	(9.1)	(41.7)	(40.8)
16:1	0.4	0.4	2.6	4.0
18:1	8.5	8.7	39.1	36.8
20:1	-	-	-	-
22:1	-	-	-	-
<u>n-6</u> esters	(41.2)	(41.0)	(27.0)	(28.8)
18:2	13.6	14.0	25.4	27.3
18:3	0.3	-	-	-
20:3	0.5	0.4	0.2	-
20:4	26.8	26.6	0.8	1.0
22:4	-	-	0.4	0.3
22:5	-	-	0.2	0.2
<u>n-3</u> esters	(19.0)	(18.7)	(0.5)	(4.8)
18:3 ^a	0.3	0.2	2.6	2.5
18:4	-	-	-	-
20:4	-	-	-	0.1
20:5	0.9	0.6	0.2	0.1
22:5	1.2	0.7	0.5	0.4
22:6	16.6	17.2	1.7	1.7
Others	1.2	1.5	2.0	1.4

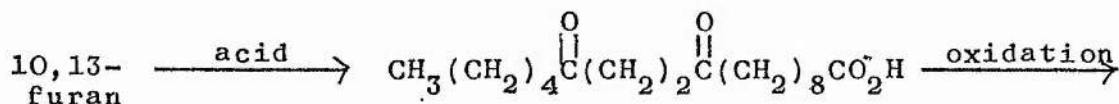
^a May contain 20:1

^b Cod liver oil

Table 20 (Cont).

Feces (10,13-furan-fed)	Liver (CLO-fed)	Adipose Tissue (CLO-fed)	CLO ^b (feed)	Rat Cake
(32.9)	(23.7)	(24.0)	(13.1)	(16.0)
0.5	0.2	1.9	3.3	3.0
18.5	13.5	18.7	8.4	10.4
13.9	10.0	3.4	1.4	2.6
(25.3)	(19.5)	(47.8)	(46.5)	(43.5)
1.0	2.0	7.7	7.6	6.0
24.3	15.7	33.9	17.6	20.1
-	1.4	5.2	13.0	10.5
-	0.4	1.0	8.3	6.9
(33.3)	(18.5)	(14.4)	(3.2)	(14.2)
28.4	12.7	13.9	1.9	12.8
1.3	-	-	-	-
-	0.5	-	-	-
1.2	5.0	0.2	-	0.4
1.6	0.3	0.2	0.6	0.4
0.8	-	-	0.7	0.6
(4.1)	(37.5)	(12.7)	(36.2)	(24.9)
3.2	0.3	1.1	0.3	-
-	-	0.6	3.5	2.5
-	-	-	0.8	0.4
0.4	8.3	2.4	13.9	10.4
-	4.8	1.5	1.1	0.9
0.5	24.1	7.1	16.6	10.7
3.8	0.8	1.2	1.0	1.4

are fed to rats products arising from similar degradation have been observed¹⁸⁹. It may be that furanoid acids do not survive the acidity of the digestive system and are ring-opened to diketones which could undergo oxidation in the normal fashion.



(b) Rats given cod liver oil

A group of three female rats were given rat cake (14 g/day) supplemented with cod liver oil (1 g/day). The rats were maintained on this diet for 15 weeks and they gained weight considerably during this period, probably because of nourishment from cod liver oil (Table 21).

Table 21: Weight gain of cod liver oil-fed rats

	<u>Start</u>	<u>Finish</u>	<u>% Gain in weight</u>
Rat 1	190	340	78.9
Rat 2	242	317	30.9
Rat 3	191	289	51.3

Average gain in weight 51.8%

Pronounced alterations were observed in the fatty acid composition of the liver and adipose tissue of rats given diets supplemented with cod liver oil (Table 20). Increased dietary availability of the n-3 acids 20:5 and 22:6 resulted in a major increase in the content of these acids in both the liver and the adipose tissue. The C_{20/22} monoene

acids which were not present in the standard group, appeared in the liver and adipose tissue after feeding with cod liver oil and the level of 18:1 in the liver increased. This increase in the proportion of monoene acids and n-3 polyene acids in the liver lipids is balanced by a marked fall in the level of arachidonic acid. The level of 18:0 also diminished while that of 16:0 remained the same.

Perhaps the more important finding in this experiment is the absence of in the liver and accumulation in the adipose tissue of F acids in the rats given cod liver oil. The cod liver oil feed contained F acids (1.6 %) and P acids (0.5%) and these two kinds of acids were present in the adipose tissue at levels of 0.6% and 1.6% respectively, (Table 22). The absence of F acids in the liver lipids is noteworthy in view of the observation by Glass et.al.¹⁹³ that when 10,13-furan acid ($2-^{14}\text{C}$) was fed to fish it accumulated in the liver and was also chain-elongated in the normal fashion. Thus, it appears that the F acids are metabolized differently by the two animals.

Only trace amounts of P acids were present in the liver lipids of cod liver oil-fed rats but the ratio between the individual P acids in the liver and also in the adipose tissue was the same as that in the cod liver oil. The ratio of individual F acids accumulated in the adipose tissue was, however, different from that in the feed, with $\text{F}_2\text{-F}_4$ accumulating in preference to the longer-chain members F_5 and F_6 (Table 22).

Although limited, these experiments suggest that the fish-furanoid acids (F_1 - F_7) are absorbed by the rat and are deposited in adipose fat. Whether the animal is able to utilize the stored F acids as a source of energy or

Table 22: GLC of furan bands^a

Acid	Cod liver oil-feed (CLO)	Adipose Tissue (CLO-fed)	Liver (CLO-fed)
P_{16}	6.0	14.4	13.3
P_{19}	4.3	11.5	11.7
P_{20}	12.1	31.7	31.2
U_1	3.7	4.7	-
U_2	?	0.4	-
F_1	-	-	-
F_2	9.5	6.4	-
F_3	1.2	2.1	-
F_4	6.8	2.9	-
F_5	9.4	2.7	-
F_6	28.0	7.3	-
F_7	1.0	0.7	-
Others	18.0	15.2	43.8 ^b
% furan band	2.2	2.6	?
% $P_{16}+P_{19}+P_{20}$	0.5	1.5	trace
% F_2-F_7	1.6	0.6	-
% U_1+U_2	0.1	0.1	-

^a The furan bands of neither the liver nor the adipose tissue of the control group showed any GLC peaks

^b Contains mainly 16:1 and 18:1

essential fatty acid cannot be ascertained at this stage. Similarly no conclusions can be drawn whether the F acids might have a deleterious effect on general body metabolism if they were allowed to reach higher concentrations in the tissues by feeding bigger ^sdoxes over longer periods. No harmful effect upon the general health of the animal has been observed when moderate amounts of F acids were fed for 15 weeks. Thus, quantities of F acids that may be encountered in natural diets are probably not deleterious to individuals consuming them for that period.

2. MAJOR ACIDS

(i) General

Total lipids were extracted from the biological samples. Sometimes these were separated into cholesterol esters, triacylglycerols and (unresolved) phospholipids but more generally the total lipids were examined. After conversion to methyl esters these were subjected to GLC with the results shown in Table 25. The identification of esters was based mainly on their chromatographic behaviour (ECL) making use of the valuable data reported by Jamieson¹⁹⁴. These conclusions were confirmed, in part, by urea fractionation though this treatment was designed to concentrate the furanoid and branched-chain acids and was not specifically optimized for the separation of overlapping monoenes and polyenes (such as 20:1 and 18:3 n-3). Some ester fractions were submitted to Ag⁺ TLC. The results supported the assignments made to the major components and also revealed the presence of minor components masked by esters present in higher proportions.

On the basis of previous reports on lipids of aquatic origin we examined our chromatographic results for saturated and monoene esters and for the n-6(18:2 to 22:5) and n-3(18:3 to 22:6) polyenes. This left some minor components among which we recognised 16:2(n-4), 20:2(n-6), methyl phytanoate, and the major furanoid acid (F₆). More careful studies of selected samples showed the presence of the additional phytol-based and furanoid esters

discussed in section I.1. We also recognised small amounts of 16:3, 16:4 and 20:3 but these are ignored in Table 25.

The biggest difficulty in making these assignments results from the overlap of C_{18} , C_{20} , C_{22} , and C_{24} monoene esters with polyenes of shorter-chain length. For example, depending on the polarity of the GLC columns employed, 18:1 may overlap with 16:3($n-3$) and/or 16:4($n-3$), 20:1 with 18:3($n-3$), 22:1 with 20:3($n-3$) and 20:4($n-6$), and 24:1 with 22:5($n-6$). We divided such peaks between their monoene and polyene components on the basis of information obtained in the urea fractionation where all the monoene and some of the polyene is present in the urea adduct and the remainder of the polyene but none of the monoene is in the mother liquor. Fractionation of cod liver oil (Table 23) is a typical example. Table 24 illustrates the calculation of the relative proportion of the urea adduct and mother liquor by consideration of the distribution of the major acids between the two fractions. The separation of 20:3($n-3$) and 20:4($n-6$) is very difficult on packed columns and we have assigned all the polyene components of this peak to arachidonate even though a detailed study in one case (powan, male) suggests the presence of both polyenes.

(ii) Component Acids of Total Lipids

Our results (Table 25) are in general accord with the broad statements quoted in the Introduction but closer inspection reveals many useful and interesting correlations. Besides confirming the well-known differences between lipids

Table 23 : Fractionation* of cod liver oil methyl esters by treatment with 5 times their weight of urea.

<u>Ident</u>	<u>Total</u>	<u>Urea Adduct</u>	<u>Mother Liquor</u>	<u>Furan Band</u>	<u>Ident</u>
?	12.50(0.1)				
?	13.24(0.1)		13.80(0.7)		
14:0+P ₁₆	14.11(3.9)	14.11(4.7)	14.22(0.9)	13.91(10.2)	P ₁₆
?	14.70(0.3)			14.87(0.5)	?
?	15.10(0.3)		15.02(0.1)		
P ₁₉	15.71(0.3)		15.75(1.3)	15.62(0.3)	P ₁₉
16:0	16.01(10.5)	16.00(13.4)		15.93(0.3)	?
16:1	16.55(8.9)	16.65(11.1)	16.60(0.2)	16.36(0.5)	?
P ₂₀	17.01(0.8)		17.07(4.4)	16.97(6.5)	P ₂₀
16:2	17.50(0.7)				
16:4+18:0	17.98(2.3)	18.00(2.5)	18.09(0.9)	17.97(0.4)	?
18:1	18.39(21.1)	18.44(27.2)		18.25(0.6)	?
18:2	19.15(1.5)	19.18(1.7)	18.98(1.1)	18.87(1.3)	U ₁
?	19.31(0.4)				
18:3(<u>n</u> -6)	19.67(0.4)		19.78(0.3)		
18:3(<u>n</u> -3)	20.10(0.9)				
20:1	20.34(12.5)	20.38(16.6)		20.44(1.3)	F ₁
18:4(<u>n</u> -3)	20.63(2.5)		20.68(9.7)	20.81(0.3)	U ₂
20:2	21.10(0.2)				
F ₂	21.28(0.2)		21.30(0.7)	21.36(10.1)	F ₂
20:3(<u>n</u> -6)	21.57(0.1)			21.62(0.2)	?
20:3(<u>n</u> -3)+ 20:4(<u>n</u> -6)	22.00(0.7)		22.01(0.7)	21.96(2.7)	F ₃
22:1	22.23(7.6)	22.27(9.8)	22.31(0.3)		
20:4(<u>n</u> -3)+F ₄	22.47(1.2)		22.45(0.2)	22.42(12.1)	F ₄
20:5(<u>n</u> -3)	22.91(9.9)	22.92(6.8)	22.92(28.9)	23.33(8.7)	F ₅
F ₆	23.76(0.5)		23.77(2.4)	23.90(43.3)	F ₆
22:4(<u>n</u> -6)	23.94(0.6)	23.94(0.2)	23.90(1.2)		
24:1+22:5(<u>n</u> +6)	24.28(0.6)	24.32(0.6)	24.34(0.2)	24.36(0.7)	F ₇
22:5(<u>n</u> -3)	24.83(1.0)		24.81(0.4)		
22:6(<u>n</u> -3)	25.29(9.9)	25.24(4.4)	25.26(45.2)		

* Results given as ECL (DEGS) and area (weight) %.

Table 24: Calculation of the Relative Proportions of the Urea Adduct and Mother Liquor by Considering the Distribution of Some Major Esters Between the Two Fractions.

<u>Ester</u>	<u>T</u> [*]	<u>UA</u> [*]	<u>ML</u> [*]	$\frac{ML-T}{ML-UA} \times 100$
16:0	10.5	13.4	-	78
16:1	8.9	11.1	0.2	79
18:0	2.3	2.5	0.9	82
18:1	21.1	27.2	-	77
20:1	12.5	16.6	-	75
22:1	7.6	9.8	0.3	77
20:5(<u>n</u> -3)	9.9	6.8	28.9	86
22:6(<u>n</u> -6)	9.9	4.4	45.2	86

*

Therefore, urea adduct is 80% of the total. T, UA and ML stand for total, urea adduct and mother liquor respectively

of marine and freshwater origin we detect a significant difference between the fatty acids of male and female of freshwater origin. (The range of samples studied do not allow us to make any claims about sex-linked differences among the component acids of marine fish).

The following points merit mention:

(a) The saturated acids (range 9-30, mean 20%) are myristic, palmitic, and stearic with the C_{16} acid always about 70% of the total. There is less variation in the content of saturated acids among the (nine) marine lipids (17-19%) than among the (seven) freshwater lipids (9-30%).

(b) Among the monoene acids (range 17-52, mean 36%), 18:1 is always large (range 10-37, mean 20%). The C_{20} and C_{22} monoenes are significant in the lipids of marine fish such as capelin (42%), herring (25%), dogfish (20%), and cod (20%) but they are only minor components in most freshwater fish (range 0.7, mean 1%).

(c) The content of $n-6$ polyene acids is higher in freshwater fish (range 9-18, mean 13%) than in those of marine origin (range 2-7, mean 5%). The $n-3$ polyene acids of (three) male freshwater fish (18-22%) are less common than those of (four) female freshwater fish (39-43%) or of lipids of marine origin (25-46%), except capelin at 11%). The $n-3$ acids (range 11-50, mean 32%) always exceed the $n-6$ acids (range 1-18, mean 8%) and the latter are higher in freshwater fish (range 9-18, mean 13%). The ratio of $n-3$ acids to that of $n-6$ is greater among the marine lipids (6-9, except for capelin where it is only 4.7)

Table 25 (cont)

species sex	ice fish m	octopus m	haddock ?	herring ?	mackerel ?	dogfish m/f	capelin m/f	cod m/f
saturated	(24.5)	(11.6)	(18.4)	(16.6)	(18.5)	(17.5)	(18.3)	(16.8)
14:0	5.5	1.8	0.7	6.5	2.0	3.0	6.9	4.0
16:0	18.0	7.3	12.7	9.1	13.1	11.8	10.3	10.5
18:0	1.0	2.5	5.0	1.0	3.4	2.7	1.1	2.3
monoenes	(34.6)	(31.2)	(19.4)	(41.7)	(33.2)	(46.4)	(65.1)	(50.8)
14:1	-	-	-	-	0.3	0.3	0.3	0.3
16:1	14.0	9.6	1.9	6.2	3.3	5.7	8.5	8.9
18:1	19.0	17.6	10.0	9.8	22.2	19.4	14.0	21.0
20:1	1.1	3.9	1.2	10.1	3.1	10.0	18.9	12.5
22:1	0.5	0.1	4.4	14.8	3.1	10.1	22.6	7.6
24:1	-	-	1.9	0.8	1.2	0.9	0.8	0.5
n-6 polyenes	(3.9)	(7.4)	(6.2)	(4.7)	(5.1)	(3.9)	(2.3)	(3.4)
18:2	2.5	0.7	1.3	2.2	2.5	1.5	1.3	1.5
18:3	0.2	0.1	-	-	-	0.5	0.2	0.4
20:3	0.1	0.1	0.3	-	0.3	0.2	0.1	0.1
20:4	1.1	5.9	2.6	0.4	0.7	0.8	0.4	0.7
22:4	-	0.2	0.3	1.5	0.6	0.7	0.3	0.6
22:5	-	0.4	1.7	0.6	1.0	0.2	-	0.1
n-3 polyenes	(29.0)	(46.0)	(50.3)	(36.0)	(43.2)	(27.1)	(10.9)	(25.4)
18:3	0.7	-	-	-	1.0	1.1	0.7	0.9
18:4	1.9	0.3	0.9	7.0	3.8	2.5	2.5	2.5
20:4	-	0.9	0.9	-	2.0	1.4	-	1.2
20:5	16.0	29.5	20.4	11.9	11.7	8.4	5.2	9.9
22:5	0.3	1.9	3.4	1.4	2.0	1.8	0.4	1.0
22:6	10.1	13.4	24.7	15.7	22.7	11.9	2.1	9.9
others								
P ₂₀ *	-	-	1.5	-	-	0.9	0.5	0.9
16:2(n-4)	2.5	0.5	-	-	-	0.7	0.6	0.7
20:2(n-6)	0.2	1.6	2.3	0.4	-	0.5	0.2	0.2
F ₆ *	1.4	0.1	-	-	-	0.5	-	0.5
unident	3.9	1.6	1.9	0.6	-	2.5	2.1	1.3
n-3/n-6	7.4	6.2	8.1	7.7	8.5	7.0	4.7	7.5
20:5 + 22:6	26.1	42.9	45.1	27.6	34.4	20.3	7.3	19.8

* P₂₀ refers to methyl phytanate and F₆ to the most common of the furan-containing acids viz 6.

This unusually high proportion of 14:0 probably represents contamination with antioxidant (BHT)

than among those of freshwater origin (1-4 for both male and female).

(d) In almost all cases the 20:5 and 22:6(n-3) acids are major components (total 12-43%) with the lower values being associated with lipids from male freshwater fish. The 22:6 content exceeds that of 20:5 except for ice fish and octopus.

(e) Among the freshwater fish we examined, samples from three males contained more monoene acids (41-45%) and less n-3 polyenes (18-22%) than those from four female (15-23% and 39-43% respectively).

(iii) Differentiation of Lipids of Freshwater and Marine Origin

Prominent differences are observed in the component fatty acids of the (seven) freshwater lipids on the one hand and the (nine) marine lipids on the other. Arachidonic acid which is the principal polyunsaturated fatty acid in the tissues of terrestrial animals also occurs at a lower level, in the freshwater lipid samples examined in this study but is virtually absent in the marine lipids. Largely due to its presence, freshwater lipids contain significantly more n-6 acids than the marine lipids and the n-3/n-6 ratio is always higher for the latter. The lipids could be categorized according to this ratio as the figures for the marine lipids (mean 7.2) are about thrice those for the freshwater samples (mean 2.5).

The majority of the marine lipids contained significantly more C₂₀ and C₂₂ monoene acids than the lipids from freshwater sources. For example the cod, dogfish,

capelin, and herring each contained 20% or more of $C_{20/22}$ monoene acids, whilst, except for the pike, none of the freshwater fish had more than 3% of these two acids. However, the icefish (1.6%), octopus (4.0%), haddock (5.6%), and the mackerel (6.2%) contained too little of these acids to merit inclusion in the category of marine lipids.

Hilditch and Williams⁷⁴, following the views of Lovern¹⁹⁵, point out that the lipids from freshwater fish, in comparison with those of marine origin, are richer in unsaturated C_{16} and C_{18} acids, with lower contents of those of C_{20} and C_{22} series. Except for the icefish, all of the distinctly marine lipids are indeed richer in the longer-chain unsaturated acids. Among the freshwater lipids, however, only those of male species fit into the scheme, with those of the females being more like marine lipids. Thus the $n-3/n-6$ ratio is the most significant feature distinguishing freshwater from marine fish lipids as previously noted by Ackman²².

The differences observed in the fatty acid composition of marine and freshwater fish can be traced back to differences in composition of dietary phytoplankton in seas and inland waters¹⁹⁶. In marine phytoplankton predominate the diatoms with low levels of linoleic and linolenic acids, in freshwater bodies, however, the green and blue-green algae with high levels of both fatty acids. The distribution of these fatty acids in the lipids of herbivorous planktonic crustaceans shows the same pattern as in their food.

Although most marine and freshwater fish are able to desaturate and elongate linoleic and linolenic acids to long-chain polyene fatty acids, the bulk of these fatty acids originate in lower trophic levels. More longer-chain fatty acids are found progressively when moving up the food chain. The major saturated and monoene acids in the phytoplankton are C_{14} and C_{16} and the polyene acids are mainly C_{16} with 2-4 double bonds¹⁹⁷. The major consumers of phytoplankton in the oceans are the herbivorous zooplankton. These differ from phytoplankton in that they have higher proportions of longer-chain units; this situation applies equally to monoene and polyene fatty acids. This trend to produce fatty acids of longer-chain length than those present in the dietary lipids started in the zooplankton is continued in the fishes, the distribution of the long-chain polyene acids in the lipids of marine and freshwater species being similar to that in their natural food¹⁹⁶.

Among the marine fish examined by us the content of saturated fatty acids showed little variation (range 17-19%) but such constancy was not observed among the freshwater samples, (range 9-30%). The proportion of palmitic acid in the total saturated acids, however, was remarkably constant (~70%), the value of the constant being slightly higher for freshwater fish.

This close similarity in saturated fatty acid composition supports the view expressed by Brenner et.al. that in fish lipids the saturated fatty acids are generally optimized at a composition of this type, revolving round

palmitic acid as the chief saturated acid in a metabolic fatty acid pool¹⁹⁸. These workers noticed a significant increase of palmitic acid in the triacylglycerols of two freshwater fish Pimelodus maculatus and Parapimelodus valenciennesi when kept on a fat-deficient diet. They attributed this increase to de novo synthesis by the fish. It was also noticed that addition of methyl palmitate to the fat-deficient diet did not modify the fatty acid composition of the lipid and did not even increase the concentration of palmitic acid. Thus the level of palmitic acid appears to be well regulated by fish and Brenner et.al.¹⁹⁸ suggested that this regulation is exercised mainly through inhibition of palmitic acid synthesis and total oxidation.

(iv) Sex-Linked Differences

We have observed a striking difference in the content of C_{20} and C_{22} unsaturated acids between the males and females of freshwater fish (Table 26). The females of roach (56%) and powan (43%) contained twice as much of these acids in their liver lipids than did the males (24 and 21%) respectively). A similar relationship exists between the sea trout pair (female, 43%; male, 28%). Only female brown trout and perch were studied but in accord with the aforementioned generalization, their liver lipids contained significantly more C_{20} and C_{22} unsaturated acids (46 and 52% respectively) than those of the male pike (34%)

Table 26:

Species	Freshwater					Freshwater-marine					marine					
	roach	powan	pike	roach	powan	brown trout	perch	sea trout	salmon	ice fish	octopus	haddock	herring	mackerel	dogfish	capelin
sex	m	m	m	f	f	f	f	m	?	m	m	?	?	?	m/f	m/f
Saturated acids	20.2	25.7	11.6	9.4	22.5	29.7	23.0	39.0	21.7	24.5	11.6	18.4	16.6	18.5	17.5	18.3
Monotene acids	44.9	40.9	43.5	21.9	23.0	17.2	15.3	33.1	52.2	34.6	31.2	19.4	41.7	33.2	46.4	64.8
n-6 acids	9.1	9.8	17.8	18.1	11.1	11.7	15.3	3.8	1.3	3.9	7.4	6.2	4.7	5.1	3.9	2.3
n-3 acids	18.4	20.5	21.5	43.3	41.6	38.7	42.8	22.9	22.9	29.0	46.0	50.3	36.0	43.1	27.1	10.9
20:1 + 22:1	2.2	-	6.6	1.2	-	0.5	-	2.6	9.0	1.6	4.0	5.6	24.9	6.2	20.0	41.5
C ₁₆	30.0	27.5	18.8	12.7	22.6	24.6	20.7	34.0	18.6	32.0	16.8	14.6	15.3	16.4	17.5	18.8
C ₁₈	37.1	46.2	41.4	23.2	30.9	24.4	22.1	34.5	45.2	25.3	21.2	17.2	20.0	32.8	27.8	19.8
C ₂₀	14.4	12.3	20.6	21.4	21.5	18.2	21.1	10.8	11.8	18.3	40.4	25.4	22.4	17.8	30.3	24.6
C ₂₂	9.4	8.7	13.6	34.5	21.7	28.2	31.5	16.9	20.8	10.8	16.0	34.4	33.9	29.3	37.3	25.4
C ₁₆ + C ₁₈	67.1	73.7	60.2	35.9	53.5	49.0	42.8	68.5	63.8	57.3	38.0	31.8	35.3	49.2	45.3	38.6
C ₂₀ + C ₂₂	23.8	21.0	34.2	55.9	43.2	46.4	52.6	27.7	32.6	29.2	56.0	59.9	56.4	47.2	67.6	50.0
n-3/n-6	2.0	2.1	1.2	2.4	3.7	3.3	2.8	6.0	17.6	7.4	6.2	8.1	7.6	8.4	6.9	4.7
(n-3) + (n-6)	27.5	30.3	39.3	61.4	52.7	50.4	58.1	26.7	24.2	32.9	53.4	56.3	40.7	48.3	31.0	13.2
C ₁₆ unsaturated	14.0	8.3	9.7	5.8	4.2	2.6	4.9	4.1	6.2	14.0	9.5	1.9	6.2	3.3	5.7	8.5
C ₁₈ unsaturated	34.5	41.9	39.0	21.6	28.2	18.6	15.9	27.8	37.3	24.3	18.7	12.2	19.3	29.5	25.1	18.7
C ₁₆ + C ₁₈ unsaturated	48.5	50.2	48.7	27.4	32.4	21.2	20.8	31.9	43.5	38.3	28.2	14.1	25.2	32.8	30.8	27.2
16:0 saturated acid x 100	79.2	74.7	78.4	73.4	82.1	74.1	68.7	76.7	57.1	73.4	63.5	68.6	54.5	70.4	67.4	63.1

It is also possible that sex-linked differences exist among the component acids of marine lipids but the range of marine fish used in this study does not permit us to draw any conclusions.

The increase of the level of C_{20} and C_{22} unsaturated acids in the liver lipids of female freshwater fish is accompanied by a corresponding decrease in the level of saturated and monoene fatty acids. The lipid contents of the male and female livers were not very different (Table 11). It follows that the female contains more long-chain unsaturated acids in absolute terms, which suggests that it has a more active system of chain-elongation and desaturation enzymes than has the male.

The higher content of C_{20} and C_{22} unsaturated acids in the female lipid samples is largely as a result of a preponderance of n-3 acids in that sex. The major polyene acids of fish lipids and lipids of other aquatic animals are predominantly of the n-3 series and by contrast those of the lipids of terrestrial animals belong to the n-6 series. For a long time, this abundance of n-3 acids in the lipids of aquatic animals was simply regarded as a means of ensuring that biomembranes retain their fluidity at low temperatures. In the past few years, however, the role of n-3 acids as essential nutrients for fish has emerged.

The dietary essentiality of n-3 acids for fish was first demonstrated by Castel and coworkers^{38,199,200} using rainbow trout. Comparing the health of several

groups of trouts fed on diets supplemented with various fatty acids other than linolenic acid, with those fed on a diet containing linolenic acid, they found that weight gain was best in the latter group. In addition, pathologies were observed in all groups except that group given linolenic acid in the food. These findings have now been amply confirmed by Watanabe et.al.²⁰¹ who also showed that longer-chain, more-unsaturated members of the n-3 series (20:5 and 22:6) have greater essential fatty acid activity for trout than does linolenic acid²⁰². Our consistent observation that the n-3 acids are significantly more abundant in female fish than in male fish suggests that the requirement of these acids is greater for the female than for the male.

(v) Some Comments on the Other Lipid Samples

The ice fish is a very unusual species living under the Antarctic ice with no red blood system. Whilst its liver lipid is typically marine in respect to its high n-3/n-6 ratio of 7.4 the content of 20:1 and 22:1 (1.6% total) is unusually low for a marine lipid. It is also somewhat atypical in the high content of saturated acids and in the fact that the proportion of 20:5(n-3) exceeds that of 22:6(n-3).

Salmon spend part of their life in rivers and part in the sea. Our sample of salmon was a mature fish caught in a river in late summer and was presumably migrating upstream to spawn. Its liver lipid was very low in n-6 acids indicating that salmon do not feed during

their spawning migration. Because of its low level of $n-6$ acids, the salmon had a high $n-3/n-6$ ratio characteristic of marine fish. As regards unsaturated acids it contained more $C_{16/18}$ acids than $C_{20/22}$ acids and in this respect it behaved like a freshwater fish.

Sea trout and brown trout also belong to the salmon family. The sea trout showed the $n-3/n-6$ ratio typical of a marine fish and differs in this respect from the brown trout. Sea trout and brown trout are the same zoological species but brown trout lives in freshwater and unlike the sea trout does not migrate upstream to spawn. Experiments have shown that if sea trout cannot get away from freshwater they change into brown trout. Also when young brown trout are introduced to the sea they grow into adult sea trout and join other trout in the upstream migration. Because of this, trout is recognised as a variable species existing in migratory (sea trout) and non-migratory (brown trout) forms²⁰³. In accord with this non-migratory character, brown trout have a $n-3/n-6$ ratio typical of freshwater fish.

(vi) Composition of Component Lipids

The component acids of the separated cholesterol esters, triacylglycerols, and phospholipids were examined for cod, roach, salmon, and octopus. A minor lipid component moving slightly ahead of triacylglycerols (TLC) was frequently observed. In roach (female) this fraction was shown to be methyl esters (R_f and direct GC). These may have been formed during storage of the livers in methanol.

Table 27: Component acids (% wt) of total lipids and of separated lipid classes

	roach (f)			salmon					octopus (m)				
	total	CE(3%)	TG(39%)	PL(58%)	total	CE(1%) ^d	CE(5%) ^d	TG(9%) ^d	TG(40%) ^d	total	CE(3%)	TG(44%)	PL(53%)
saturated	(9.4)	(-)	(15.1)	(17.3)	(21.7)	(39.1)	(6.7)	(33.9)	(22.9)	(11.6)	(3.7)	(10.8)	(12.1)
14:0	0.9	-	1.0	0.7	1.4	1.7	0.7	1.5	1.9	1.8	0.5	2.1	2.2
16:0	6.9	-	12.7	12.2	12.4	14.9	4.6	17.8	11.8	7.3	1.3	6.9	7.7
18:0	1.6	-	1.4	4.4	7.9	22.5	1.4	14.6	9.2	2.5	1.9	1.8	2.2
monoenes	(21.9)	(16.5)	(59.6) ^c	(24.9) ^c	(52.2)	(47.6)	(52.8)	(61.2)	(67.7)	(31.2)	(24.1) ^e	(31.8)	(33.6)
14:1	-	-	-	-	-	-	-	-	-	-	-	-	-
16:1	5.8	1.1	15.7	5.8	6.2	7.4	4.5	5.7	9.4	9.6	2.9	10.2	10.4
18:1	14.9	11.5	37.5	17.0 ^c	36.7	33.3	27.5	40.1	46.7	17.6	11.3	17.1	18.6
20:1	1.0	3.9	6.4 ^c	2.1 ^c	5.6	4.2	12.7	8.3	6.9	3.9	6.8	4.5	4.6
22:1	0.2	-	-	-	3.4	2.5	7.8	6.1	3.9	0.1	1.7 ^e	-	-
24:1	-	-	-	-	0.3	0.2	0.3	1.0	0.8	-	1.4 ^e	-	-
n-6 polyenes	(18.1)	(16.4)	(9.4)	(21.8)	(1.3)	(0.4)	(0.9)	(0.3)	(0.8)	(7.4)	(6.4) ^e	(9.0)	(8.9)
18:2	1.7	0.6	4.8	1.6	0.3	0.3	0.2	0.1	0.5	0.7	0.6	1.3	1.1
18:3	0.1	-	-	-	-	-	-	-	-	0.1	-	-	-
20:3	0.7	6.2 ^a	0.3	0.5	-	-	-	-	-	0.1	0.3	0.2	0.1
20:4	13.2	4.3 ^b	3.7	16.1	0.7	0.1	0.7	0.2	0.2	5.9	5.2	6.4	6.7
22:4	-	-	0.3	0.6	-	-	-	-	0.1	0.2	0.3	0.5	0.5
22:5	2.4	5.3	0.3	3.0	0.3	-	-	-	-	0.4	- ^e	0.6	0.5
n-3 polyenes	(43.3)	(9.9)	(12.4) ^c	(34.7) ^c	(22.9)	(12.6)	(29.6)	(3.6)	(7.7)	(46.0)	(61.6)	(45.1)	(41.9)
18:3	4.9	-	-	-	0.3	0.3	0.3	0.2	0.3	-	0.1	-	-
18:4	-	-	-	-	-	-	-	-	-	0.3	1.0	-	0.4
20:4	0.5	1.3	0.7	0.2	-	-	-	-	-	0.9	2.0	0.8	0.6
20:5	6.0	4.4	4.6	4.6	5.5	3.3	10.2	0.7	1.9	29.5	30.1	28.1	24.8
22:5	2.6	-	1.3	2.6	3.0	2.6	9.2	0.7	1.7	1.9	2.7	2.0	2.2
22:6	29.3	4.2	5.8	27.3	14.1	6.4	9.9	2.0	3.8	13.4	25.7	14.2	13.9
others*													
P ₂₀	-	2.4	-	-	0.5	0.2	0.3	0.9	0.4	-	-	-	-
16:2(n-4)	0.2	-	0.8	0.4	0.2	0.1	0.3	0.1	0.2	0.5	0.3	0.5	0.6
20:2(n-6)	1.3	3.6 ^b	0.7	0.9	0.1	-	0.4	-	0.1	1.6	1.0	1.8	1.1
F ₆ *	2.9	36.2 ^b	0.7	-	1.0	-	4.8	-	-	0.1	0.2	-	1.0
unident	2.9	15.0	1.3	-	0.1	-	4.2	-	0.2	1.6	2.7	1.0	0.8

see footnote to Table 2.

May also contain some furan acid (3)

c In these analyses no distinction was made between 20:1 and 18:3

d The total lipids were separated, in the order shown, into two cholesterol ester

e In this analysis no distinction was made between 24:1 and 22:5(n-6) and the

Sometimes this fraction was due to relatively more saturated TG but in all cases it was only a minor component and is omitted from Table 27.

Although there are differences between the composition of each lipid class within a single fish these are not consistent through the four samples and no clear pattern emerges. In the roach, for example, monoene acids are enriched in the triacylglycerols (i.e. a significantly higher proportion than in the total lipids) whilst the n-6 and n-3 polyenes are diminished. In general the cholesterol esters differ most from the total lipids: in the fed cod the n-6 polyenes are enriched, in the octopus the n-3 polyenes are enriched and the saturated acids diminished, and in the roach there is a concentration of F acids balanced by an absence of saturated acids and a reduction in the n-3 polyenes.

3. INVESTIGATION OF FOUR COMMERCIAL FISH MEALS

Introduction

Fish meal in the broadest sense means any dry, powdered product made from vertebrate or invertebrate fish or fish offal. It is used in the feeding of animals primarily because of its protein content, but it contributes also to a substantial degree to the mineral and lipid nutrition of the animal²⁰⁴.

Almost all of the fish that are currently converted to fish meal are the so-called industrial fish such as menhaden, sand-eel, anchoveta and pout which are presently unmarketable in large quantities as human food. Apart from unpalatability, these fish are not used for human consumption because they are too small or break down or turn rancid too quickly for economic storage and subsequent heading, gutting, cleaning and processing²⁰⁵. Conversion of these unacceptable species to fish meals to feed animals, subsequently consumed by man is obviously a good method of obtaining edible protein.

In view of the fact that fish lipids generally contain furanoid acids, the toxicity of which is unknown, we examined four commercial fish meals which are popular feeds for chickens (broilers and layers), turkeys, pig and also for farmed fish. Besides searching for furanoid acids, we were also interested in the quality and quantity of the major component acids of the meal lipid. The latter is a good source of metabolizable energy and, in addition, contain acids essential for the general health of an

animal. Although the term essential fatty acid is normally applied to acids of the n-6 family such as linoleic and arachidonic there is evidence that for many purposes, if not all, these may be replaced by the n-3 polyenes which characterize fish lipids²⁰⁶. Too much of the n-3 acids is however, undesirable because of the risk of imparting a fishy off flavour to poultry meat²⁰⁴.

Results and Discussion

The four fish meal samples were examined for F esters according to the usual procedure of urea fractionation followed by Ag⁺ TLC. Our results are set out in Table 28, along with comparable results from dogfish liver oil. The 'furan band' from this last source, representing about 1.5% of the total esters, shows peaks corresponding to the P esters (total 21%) and F esters (total 61%). Similar fractions from each of the fish meal esters represent only 0.5% of the total esters (possibly 1% for the white fish meal) and contain relatively more of the P esters (61-80%). F esters are absent or present at a level below 0.2%. Though unable to identify all the minor components of these small fractions we consider that the ester of ECL 18.19-18.35 (1.2-2.4%) is probably 18:1, and the esters at 18.57-18.81 (5.4-10.1%) and at 20.58-20.61 (0-3.5%) are U₁ and U₂ respectively.

There is some argument about the best method of estimating fat in fish meal. Extraction with diethyl ether is recommended by the International Association of

Fish Meal Manufacturers (IAFMM) whilst the EEC method involves ether extraction after acid hydrolysis. The latter procedure gives a higher lipid content, presumably because simple ether extraction does not satisfactorily extract phospholipids which are believed to comprise about 30% of fish meal lipid. We used chloroform-methanol extraction in our earlier studies of lipids from wet samples from fish livers etc. and have used the same method with fish meals (after addition of water to obtain the correct solvent ratio) believing that this gives a more complete extraction of neutral and polar lipids. The herring meal had most lipid (11%) suggesting that it contained more pelagic fish, i.e. fish where the fat is dispersed throughout the body, than in the other meals.

The Bermuda meal esters were separated by Ag^+ TLC into four fractions containing (i) saturated and monoene esters, (ii) diene esters only, (iii) triene, tetraene, and pentaene esters, and (iv) tetraene, pentaene, and hexaene esters. Some of these fractions were re-examined after catalytic hydrogenation. This study confirmed the assignments made in Table 29 and also indicated the presence of small amounts of 16:2 (part or all of the peak at 17.64), 16:3 (part of the peak assigned to 18:1), 16:4 (part of the peak assigned to 18:2), and 20:2 (part of the peak assigned to 18:4). It is likely that these minor components are also present in the other fish meals and reduce slightly the quantitative assignments made neglecting these esters.

Table 29: Component esters (% wt) of total lipid extracted from fish meal

	White fish	S. African	Herring	Bermuda
extracted lipid (%)	5.6	5.5	13.0	5.7
saturated esters				
14:0	3.2	2.8	3.2	4.0
16:0	11.1	12.9	10.3	13.5
18:0	1.7	2.9	0.6	3.7
sub-total	(16.0)	(18.6)	(14.1)	(21.2)
monoene esters				
16:1	6.8	4.6	7.0	4.7
18:1 ^a	16.9	20.2	11.5	9.7
20:1	9.7	4.7	10.3	1.8
22:1	9.1	4.2	8.7	2.4
24:1	0.9	1.2	0.6	0.9
sub-total	(43.4)	(34.9)	(38.1)	(19.5)
6 polyene esters				
18:2 ^a	1.2	4.5	1.9	3.1
18:3	-	-	0.3	-
20:3	0.2	-	0.6	0.3
20:4	1.2	1.5	-	2.3
22:4	0.5	0.3	0.5	0.8
22:5	0.3	0.6	0.3	0.3
sub-total	(3.4)	(6.9)	(3.6)	(6.8)
3 polyene esters				
18:3	-	-	-	0.3
18:4 ^a	2.4	0.5	3.3	1.9
20:4	-	0.1	0.3	0.6
20:5	12.0	9.3	18.0	18.3
22:5	1.9	2.7	0.9	3.4
22:6	19.2	25.9	19.1	25.5
sub-total	(35.5)	(38.5)	(41.6)	(50.0)
unidentified ^b				
	14.84 0.3	15.14 0.3	15.01 0.2	15.12 0.3
	17.01 0.6	16.99 0.4	17.26 0.5	17.07 0.6
	17.61 0.4	17.44 0.3	17.60 0.5	17.64 0.6
	23.36 0.2		23.37 0.4	
	23.73 0.2	23.66 0.1	23.56 0.4	23.68 0.2
			25.49 0.2	25.62 0.3
			25.93 0.1	
			26.37 0.3	26.49 0.3
				27.02 0.2
sub-total	(1.7)	(1.1)	(2.6)	(2.5)

^a The glc peak represented by these esters may contain other minor components also (see text)

^b Reported in the form equivalent chain length and % wt.

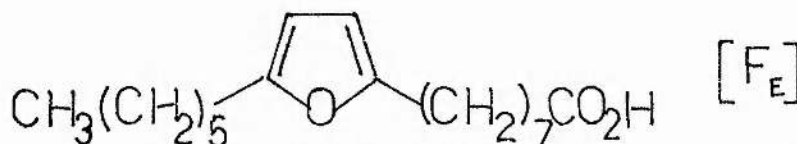
Finally we comment on the ^aquality and quantity of the esters derived from the four fish meals. All of them have a high content of n-3 esters (36-50%) with 20:5 (9-18%) and 22:6 (19-26%) predominant. The n-6 polyene esters (3-7%) are minor components. The monoene (20-43%) and saturated esters (14-21%) are more significant with 16:0 (11-14%), 16:1 (5-7%), and 18:1 (10-20%) consistently present in high proportions. The higher monoenes (C₂₀-C₂₄) are present in more variable concentrations. The Bermuda fish meal differs most from the other three with its high level of n-3 polyene and saturated esters and its very low proportion of monoene esters.

The IAFMM have supplied the following information about these fish meals. White fish: consists mainly of offal from white fish (cod, plaice etc.) processing. South African: mainly anchovy and pilchard. Herring: unlikely to contain large amounts of herring, but rather horse mackerel, mackerel or capelin if of Norwegian origin, or Norway pout or sand eel if of Danish or U.K. origin. Bermuda: produced on a factory ship and of a variable type, but normally mainly sardine or anchovy.

4. INVESTIGATION OF EXOCARPUS CUPRESSIFORMIS SEED OIL

Introduction

Morris and coworkers were the first to isolate a furanoid fatty acid from a natural source when they obtained the C₁₈ acid (F_E) from Exocarpus cupressiformis seed⁹. This compound was present in the total methyl esters at a level of 11.0% and was accompanied by several



other acids which were not identified. Later, Grundwell and Cripps¹⁷⁷ demonstrated that hydroxy enynes containing the ^{cis} unit $-\text{CH}(\text{OH})\text{CH}=\text{CHC}\equiv\text{C}-$ rearrange to furans in the presence of base or even during Ag⁺ TLC and wondered whether Morris's acid was an artefact. Because of this uncertainty we examined two samples of Exocarpus cupressiformis seed oil with the emphasis on furanoid and hydroxy enyne acids.

Results and Discussion

Ripe seed were obtained from Sydney, Australia, where this plant is common and is known as the native cherry or Cherry Ballart. The tree grows to a height of thirty feet and has cypress-like foliage. The nut is small and ovoid and is attached to a swollen red stalk. This plant is a member of a genus of some 17 species of the Santalaceae family many of which have been shown to contain enyne and hydroxy enyne acids¹⁰.

Methyl esters prepared from the seed oil by acid as well as base catalysis were examined for F. acids by the usual method of urea fractionation followed by Ag^+ TLC but neither gave a positive result. The TLC fraction corresponding to the normal furan band contained no esters at all.

Composition of the total esters is set out in Table 30. Santalbic acid (ECL 21.9, 57%) was the main component acid of the oil, followed by oleic acid (27%). Next highest was a component of ECL 24.7 (6%) followed by stearolic acid (3%). Silylation of the total esters produced a new peak at ECL 22.4 (5%) which we identified as the 8-hydroxy derivative of santalbic acid on the basis of the following results.

When the total esters were crystallized with 5 times their weight of urea, the component of ECL 22.4 (as OTMS ether) was retained in the mother liquor. It had the polarity expected of a hydroxy ester and was well separated from the esters forming adducts. Prep TLC of the liquor afforded the pure compound [fraction C, ECL 27.1; 22.3 (after silylation)]. An intense band at 3430 cm^{-1} in the IR showed the presence of a hydroxy group and a weak signal at 2200 cm^{-1} and a strong, sharp peak at 950 cm^{-1} indicated the presence of a conjugated trans-enynne moiety. Further evidence for such a enyne structure was found in the UV spectrum which had the maximum at 229 nm ($\log \epsilon = 4.21$)²⁰⁷. The hydroxyl group was assigned to C-8 on the basis of the evidence obtained by mass spectrometry of the perhydro derivative of C (as OTMS ether). This

Table 30: GLC analysis^a of total esters of Excarpus cupressiformis seed oil.

<u>Identification</u>	<u>Acid</u> ^b	<u>Base</u> ^c		<u>Total (A) (OTMS)</u> ^e
	<u>Total (A)</u> ^d	<u>Total (A)</u>	<u>Total (B)</u> ^d	
16:0	16.02(1.8)	16.07(1.4)	16.05(1.3)	15.99(1.6)
16:1	16.41(0.3)	16.43(0.2)	16.43(0.2)	16.43(0.3)
18:0	18.07(0.7)	18.10(0.5)	18.00(0.7)	18.00(0.7)
18:1	18.41(27.2)	18.43(23.7)	18.44(24.2)	18.29(25.9)
?	18.97(0.6)	18.99(0.8)	19.01(0.8)	18.99(0.9)
18:1(9a)	19.82(3.1)	19.84(2.7)	19.86(2.6)	19.78(2.5)
?	20.40(1.5)	20.41(0.6)	20.44(0.8)	20.36(0.6)
18:2(9allt)	21.97(57.0)	21.98(62.8)	22.00(58.2)	21.95(56.9)
8-OTMS 18:2 (9allt)	-	-	-	22.36(4.8)
?	-	22.93(0.6)	22.96(0.6)	22.94(0.6)
18:3(9allal3t)	24.36(7.5)	24.37(6.4)	24.47(8.2) 26.91(0.9)	24.37(5.2)

a Analysis on a DEGS column, results expressed in the form ECL and area (wt %).

b Methyl esters prepared by H_2SO_4 -catalysis

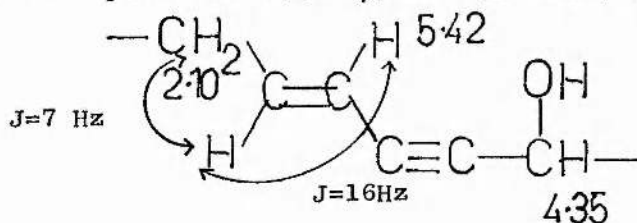
c Methyl esters prepared by NaOMe-catalysis

d A and B refer to two different samples

e GLC of the total esters after silylation

assignment was confirmed by the mass spectra of the OTMS ether of C and that of C itself (see Experimental Section).

Exocarpus cupressiformis seed oil contains santalbic acid (18:1 9allt) as the major component acid⁹ and it is assumed that C also has the 9allt structure. This is supported by the close similarity of the PMR spectra of C and santalbic acid. The doublet (J=16 Hz) at 5.42 in the PMR spectrum of C is assigned to the hydrogen on C(11) and the double triplet at 6.07 (J=16 Hz and 7 Hz respectively)



is assigned to the hydrogen on C(12). This hydroxy acid co-occurs with santalbic acid in Ximenia caffra oil²⁰⁸.

Fractions A and D were not identified. Fraction B (ECL 24.3) showed UV absorption maxima at 214, 227, 237, 253, 267, and 281. On this evidence we suspected that B is 18:3(9allal3t) because this compound has been isolated from the roots of Exocarpus cupressiformis plant²⁰⁹. B was concentrated in the urea adduct but its purification proved difficult. The mass spectrum of B showed the molecular ion at 290, and major fragment ions at 133, 91, 83, and 79 were consistent with the 18:3(9allal3t) structure.

The stalks of the seed were also examined but they contained mainly keto compounds, which were not characterized.

The absence of furanoid acids in our two samples of Exocarpus cupressiformis seed oil rekindles doubts raised by Crundwell and Cripps¹⁷⁷ whether Morris's acid (F_E) is genuine. Although hydroxy enynes readily rearrange to furans, the double bond has to be of cis geometry and located between the hydroxyl and alkyne groups for cyclization to occur. The hydroxy enyne that we found in this oil, namely 8-OH 18:1(9allt) does not have the correct structure for furanization.

Section II: SYNTHESIS

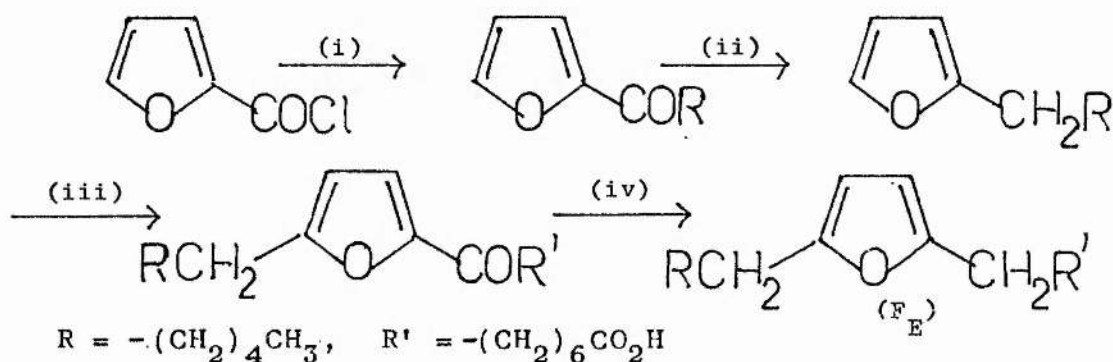
1. GENERAL INTRODUCTION

Methods of preparing furanoid fatty acids fall into two categories:

- (i) synthesis from furan or a derivative of furan,
- (ii) synthesis by chemical modification of natural acids.

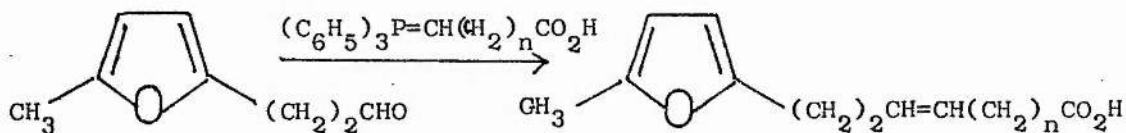
(i) Synthesis from Furan or a Furan Derivative

Elix and Sargent²¹⁰ prepared the C₁₈ furanoid acid (F_E) which was earlier isolated by Morris *et.al.*⁹ from *Exocarpus cupressiformis* seed oil. They started from 2-furoic acid and the reaction scheme is shown below.



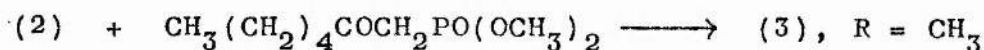
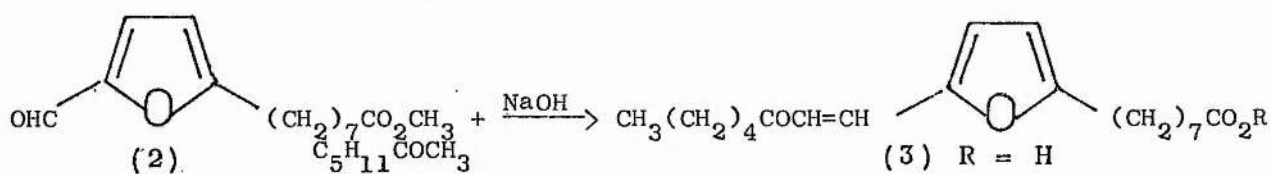
- (i) R₂Cd
- (ii) & (iv) NH₂ NH₂/NaOH
- (iii) suberic anhydride / BF₃-Et₂O

A group of Japanese workers used the Wittig reaction to prepare a series of long-chain acids (1) containing the 2,5-disubstituted furan ring system. This method was the basis of a successful patent application²¹¹.

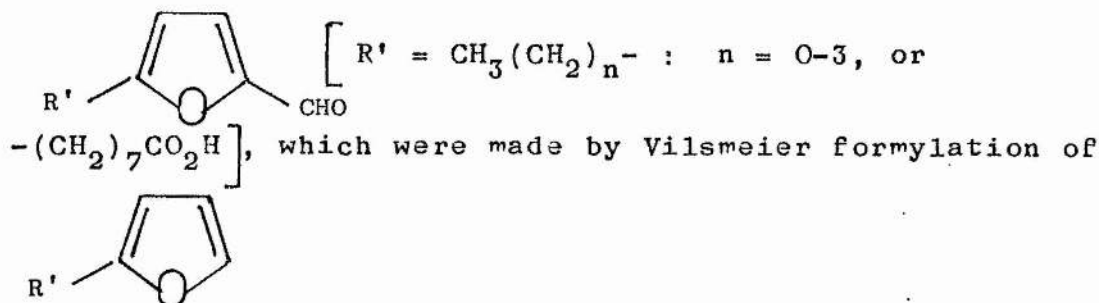


(1) n = 1-4

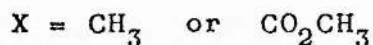
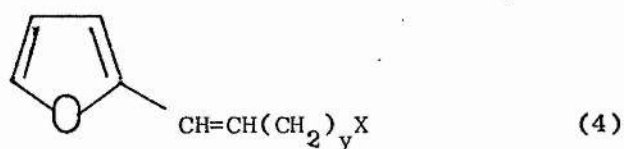
Nedenskov and Alster¹³ condensed the aldehyde (2) with an alkan-2-one or its dimethyl-phosphoric acid ester to obtain furanoid acids containing an oxo-alkenyl group, which were then modified to a variety of furanoid acids with keto and hydroxy groups. The compounds of this invention were claimed to have useful biological properties similar to that of the prostaglandin $\text{PGF}_{2\alpha}$.



The furan-aldehydes (2) used for this condensation were

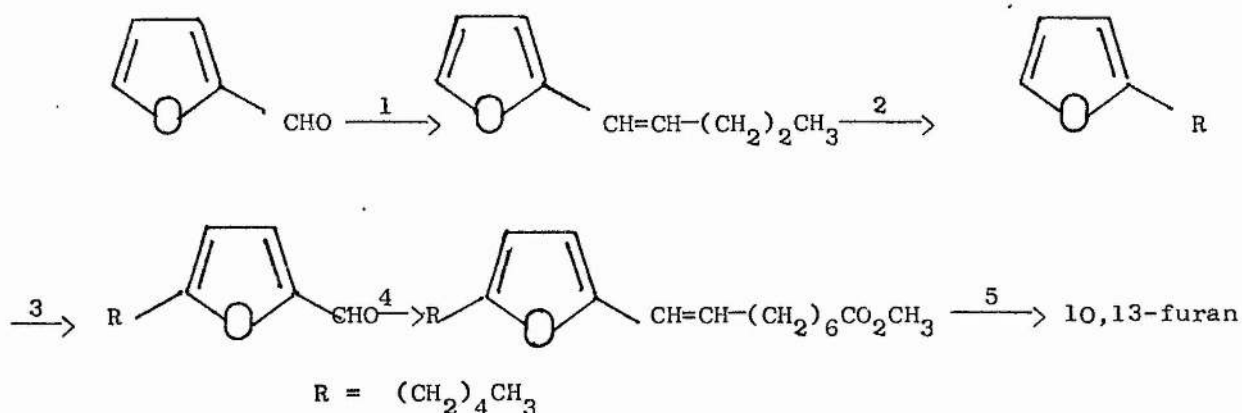


Very recently Lie Ken Jie and Lam²¹² prepared all the isomeric C_{18} furanoid acids (2,5-disubstituted) from furan or furfural. Coupling of the triphenylphosphonium salt of the appropriate 1-bromoalkane or methyl ω -bromo-alkanoate with furfural gave mainly the cis-alkenylated furan derivative (4). Catalytic hydrogenation of (4)



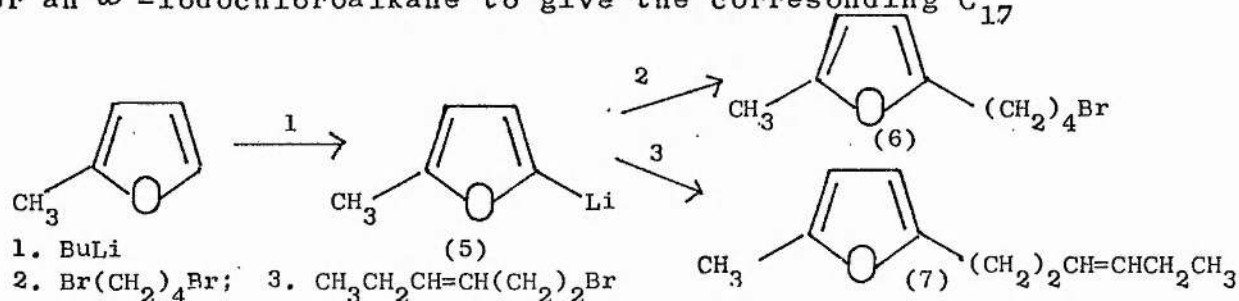
Y = number of methylene groups

using Lindlar catalyst⁵ gave the corresponding 2-alkyl furan and Vilsmeier formylation of this compound provided an aldehyde function at the 5-position of the furan ring. Chain-extension by Wittig reaction followed by hydrogenation of the resulting intermediate furnished the required C₁₈ furan ester. Preparation of 10,13-furan by this method is outlined below.

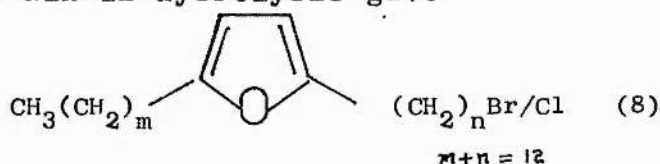


1. $\text{Ph}_3\text{P} = \text{CH}(\text{CH}_2)_2\text{CH}_3$, NaH (in DMSO)
2. H_2 , Lindlar catalyst (in ethyl acetate)
3. POCl_3 , DMF
4. $\text{Ph}_3\text{P} = \text{CH}-(\text{CH}_2)_6\text{CO}_2\text{CH}_3$, NaH
5. H_2 , Lindlar catalyst

The enhanced reactivity of the 2- and 5-positions of the furan ring lends itself to alkylation at these positions via the corresponding lithium derivatives, and this reaction has been used for the preparation of alkyl furans substituted at 2- and 5-positions²¹³. For instance, Büchi and Wüest obtained compounds (6) and (7) from the lithium derivative (5) of 2-methylfuran²¹⁴. Lie Ken Jie and Lam²¹² condensed 2-furyllithium with the appropriate 1-bromoalkane and the resulting 2-alkylfuran was similarly alkylated at the 5-position with a ω -dibromoalkane or an ω -iodochloroalkane to give the corresponding C₁₇

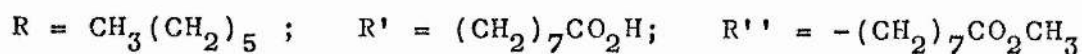
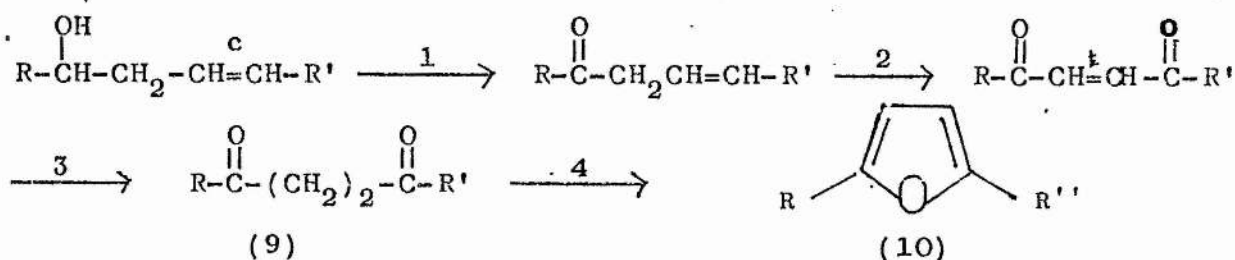


furan-containing halide (8). Conversion of the latter to the cyano derivative followed by alkali hydrolysis gave the required C₁₈ furanoid acid.



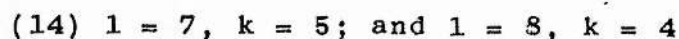
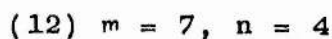
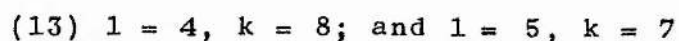
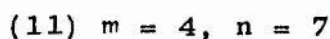
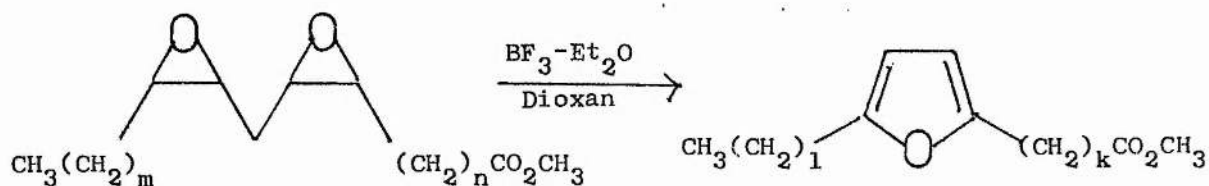
(ii) Partial Synthesis

Ring closure involving elimination of water between the dienolic forms of 1,4-dicarbonyl compounds represents one of the oldest method for the preparation of furan derivatives²¹⁵. Abbot and Gunstone²¹⁶ achieved a synthesis of the 9,12-furan (10) by cyclisation of the diketone (9) which was prepared from methyl ricinoleate according to the following scheme.



1. CrO_3
2. CrO_3 (further oxidation)
3. H_2 , Pd/C
4. $\text{BF}_3\text{-MeOH}$

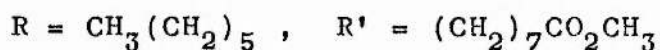
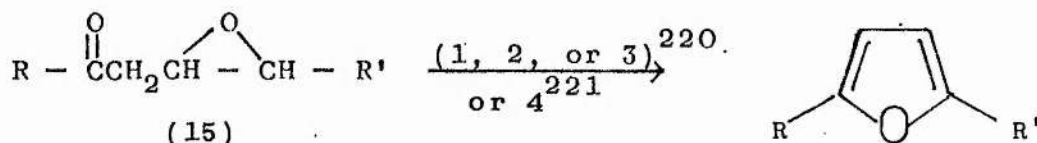
Gunstone et.al.^{217,218} have also shown that furans can be obtained by acid-catalysed reaction of cis-1,2, cis-4,5-diepoxydes. Treatment of methyl cis-9,10, cis-12,13-diepoxyoctadecanoate (11) with boron trifluoride etherate in dioxan, i.e. under conditions where epoxides usually undergo rearrangement to ketones, gave an isomeric mixture of 9,12- and 10,13-furans in approximately equal amounts²¹⁷ (over-all yield 21%). Similar reaction of methyl



cis-6,7, cis-9,10-diepoxyoctadecanoate (12) afforded a mixture of 6,9- and 7,10-furans in 33% yield²¹⁸.

Epoxides are readily isomerized to ketones by

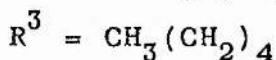
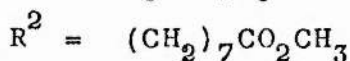
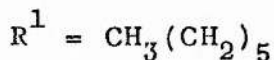
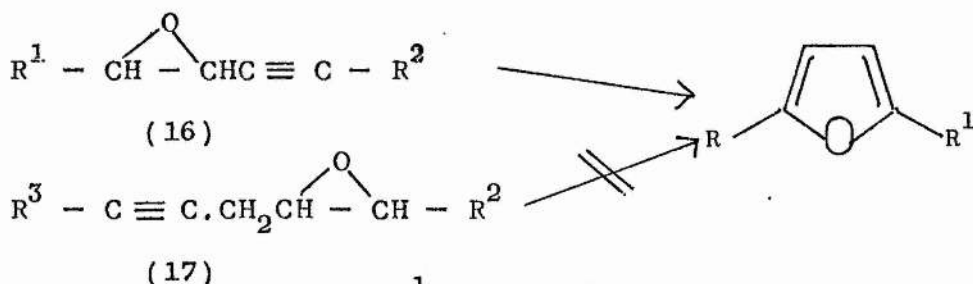
reaction with propyl iodide and sodium iodide in DMSO²¹⁹. Recently Lie Ken Jie and Lam²²⁰ used this method in an attempt to prepare methyl 9,12- and 10,13-dioxostearate from the diepoxides of methyl linoleate but obtained a mixture of 9,12- and 10,13-furans instead. Investigating this reaction further the same workers found that methyl cis-9,10-epoxy-12-oxooctadecanoate (15), when reacted under the same conditions, gave the 9,12-furanoid ester (F_B) in good yield (48%).



1. PrI, NaI, DMSO
2. BF₃-ether
3. p-toluenesulphonic acid
4. HCl, CHCl₃

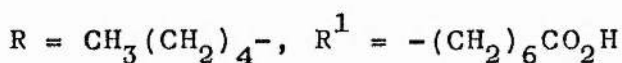
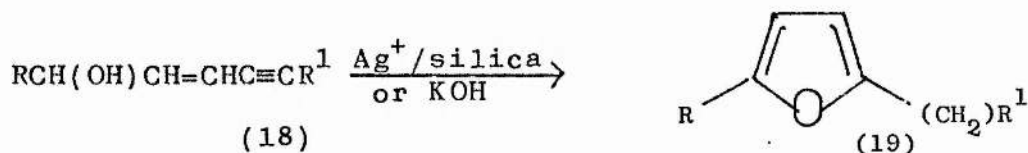
The cyclodehydration of the epoxy ketone was also achieved with boron trifluoride etherate or p-toluenesulphonic acid. Ranganathan et.al.²²¹, working independently, obtained a better yield (80%) of the ester (F_B) by using hydrochloric acid to catalyse the furanization.

In 1969, Miller²²² described the formation of furans from acetylenic $\alpha\beta$ - and $\beta\gamma$ -epoxides by hydration with dilute acid containing a catalytic amount of mercuric sulphate, and Abbot and Gunstone²¹⁷ employed this method to prepare the 9,12-furanoid ester from the epoxide of methyl ximenynate (16). The $\beta\gamma$ -epoxide (17), prepared from methyl crepenynate, however, did not furnish a furan



in their hands.

Action of base on hydroxy-enynes containing the grouping $-\text{C} \equiv \text{C} - \overset{\text{C}}{\text{C}} = \text{CH}(\text{OH}) -$ yields furans²²³. Crundwell and Cripps found that the acid (18) cyclizes to furanoid acid (19) on treatment with alkali or simply on chromatography on argentous silica gel¹⁷⁷. This cyclization of



hydroxy-enynes has also been achieved by hydration with dilute acid in the presence of mercuric sulphate catalyst, although the yields for long chain furans were rather poor²²⁴.

2. SOME GENERAL POINTS ABOUT THE SYNTHESIS OF FURANS

Although many natural products containing furan are known and have been isolated, facile methods to prepare furans are extraordinarily scarce. In spite of the advent of powerful physical methods of analysis, the

confirmation of the structure of a natural product, especially of the many exotic molecules where furan is present, still depends on the laboratory synthesis of the compound. For this reason and for many other purposes like the study of chemical and biological properties of the material, it is necessary to have at hand convenient procedures for the preparation of these compounds.

The classical procedure, namely, cyclodehydration of 1,4-dicarbonyl compounds, appears to be the only general method still available for the synthesis of furans. Although this is an equilibrium process, complete conversion of a sterically unhindered diketone to the corresponding furan may be obtained by removal of the product from the reaction medium. However, the utility of this simple conversion is severely restricted by the inaccessibility of the starting 1,4-diketones. The only commercially available 1,4-diketone is (the simplest possible one) 2,5-hexanedione (acetylacetone). The acetoacetic ester based process by which this compound is made breaks down when applied to higher homologs⁴², and cyclopentenones become the principal products instead of 1,4-diketones²²⁵.

In addition to cyclization giving furans and cyclopentenones by reactions catalysed by acids and bases respectively, 1,4-diketones undergo Knorr-Paal condensations to form pyrroles and thiophenes, and therefore have great synthetic utility. As a result, development of new synthetic routes to 1,4-diketones has received considerable attention during the past few years. The majority of the novel methods

involve coupling via carbanions or radicals, of ketone (i) with itself²²⁶⁻²²⁹, (ii) with another ketone⁵ or ketone derivative^{230,231}, (iii) or with a molecule containing functions such as epoxy^{232,233}, alkyne²³⁴ and nitro^{235,236} which are convertible to carbonyl groups. The other miscellaneous methods utilized for the preparation of 1,4-dicarbonyl compounds include coupling of dimetalloacetylides to aldehydes²²⁵, condensation of enol lactones with ynamines²³⁷, rearrangement of cyclopropyl ketones²³⁸ and reactions employing dithianes as acyl anion equivalents²³⁹.

In spite of the wide choice possible and many versatile reagents available, these methods have produced compounds of the type $\text{CH}_3(\text{CH}_2)_n\text{CO}(\text{CH}_2)_2\text{COCH}_3$ only, where $n \leq 5$. The naturally occurring furanoid fatty acids that have been discovered up to the present time have at least 18 carbon atoms and contain the furan ring in the middle of the chain. Preparation of suitable 1,4-dicarbonyl precursors for these furanoid acids is difficult, if not impossible from the presently available methods.

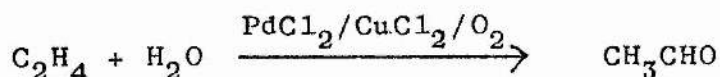
Although other methods are known for the synthesis of furan derivatives from non-furanoid precursors such as the cyclization of (i) hydroxy-enynes²²³ (ii) $\alpha\beta$ - and $\beta\gamma$ -epoxy-alkynes^{222,240} (iii) $\beta\gamma$ -epoxy-ketones²⁴¹, $\beta\gamma$ -epoxy-acetals²⁴² (iv) $\alpha\beta$ -unsaturated epoxides²⁴³ and (v) methoxyallenes²⁴⁴, they lack generality and are often inefficient. Application of these routes to the preparation of long-chain furans also suffers from the lack of suitable starting materials.

An entirely different approach involves the synthesis from furan or a simple derivative of furan. Apart from furan and furfuraldehyde these materials are not readily available and although they are applicable to a good number of syntheses, quite generally, the procedures are rather involved and yields are often low.

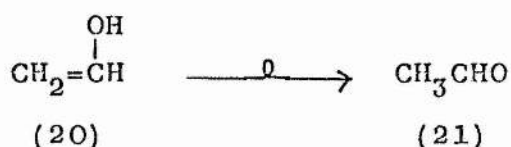
In the case of long-chain furanoid compounds, it is usually more convenient to prepare them from suitable naturally occurring precursors rather than by total synthesis. The aim of the present study was to discover novel methods to carry out such conversions.

3. SYNTHESIS INVOLVING Pd(II)-CATALYSIS

In the past fifteen years considerable advances have been made in homogeneous catalysis in general and Pd(II) catalysis in particular. Undoubtedly, much of the impetus for syntheses involving Pd(II) catalysis comes from the Wacker process for manufacture of acetaldehyde from ethylene. This process, disclosed by Smidt and coworkers²⁴⁵ in 1959, is now the preferred method of manufacturing acetaldehyde.



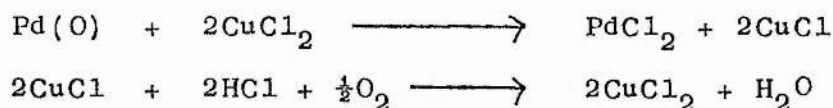
This conversion may be envisaged as initial hydration of the olefin to produce the enol (20) followed by rearrangement to the aldehyde (21).



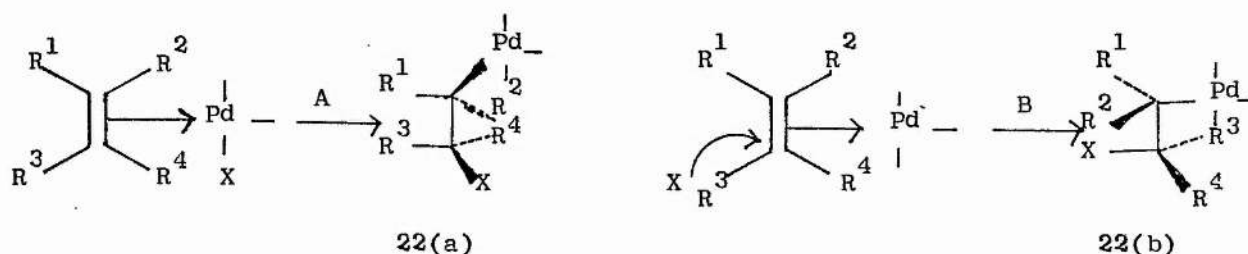
Substitution of alkenes with nucleophiles to obtain products such as (20) cannot normally be accomplished because the π -character of the carbon-carbon double bond favours the electrophilic addition ~~of nucleophiles~~. Nevertheless, coordination of the alkene to certain transition metal ions, especially Pd(II), activates the double bond so that nucleophilic groups such as hydroxy, alkoxy, and amino can react with it to give the corresponding substituted alkene²⁴⁶.

There are several reasons for the importance of Pd(II)-promoted reactions. First, the olefin π -complex^P does not need to be isolated during the reaction, but is formed as a reactive intermediate when the alkene is introduced into the Pd(II)-containing solution^{247,248}. Secondly, Pd(II) can readily expand its co-ordination sphere to accept a fifth and sixth ligand. This may allow the incoming nucleophile to co-ordinate to the metal ion before attacking the olefin, thus lowering the activation energy of the nucleophilic attack²⁴⁸. Thirdly, the palladium-olefin bond is usually weak so that any rearrangement process that may be necessary during the course of the reaction is feasible²⁴⁸. Finally, the reaction may often be carried out catalytically^{247,248} When the reaction of the co-

ordinated olefin with the nucleophile comprises an oxidation process. Pd(II) is reduced to give Pd(0), but in many cases a continuous reoxidation of the metal is possible by adding an oxidizing agent such as cupric chloride or benzoquinone



Although it is now reasonably clear that unstable intermediates of type (22) containing Pd-C σ -bonds are involved, the exact steric course of the reaction is less well understood. The mechanism of the Wacker process

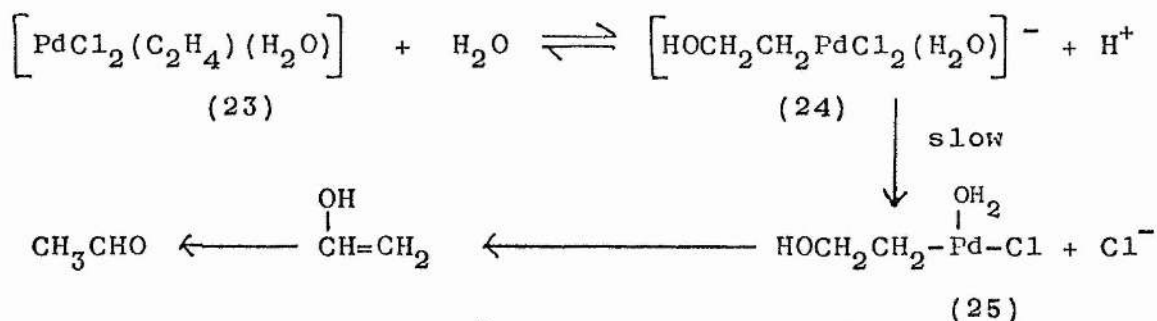


X = Nucleophile

itself has attracted considerable interest,²⁴⁹⁻²⁶⁰

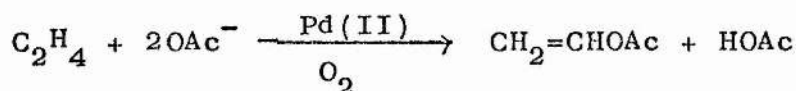
Kinetic studies of the Wacker reaction suggest that a cis-attack upon the carbon-carbon double bond, of Pd(II) and co-ordinated OH takes place²⁴⁹⁻²⁵³ (path A). A few Pd(II)-promoted reactions have in fact been shown to occur in this manner²⁵², but some reactions related to the Wacker hydroxylation, e.g. acetoxylation²⁵², methoxylation^{254,255}, and amination^{256,257} of mono-alkenes proceed in a trans manner (Path B).

Conclusive evidence that hydroxypalladation in the Wacker process occurs in a trans manner is now emerging²⁵⁸⁻²⁶⁰. On the basis of their results for the stereochemistry of the hydroxypalladation^{at}, Bäckvall et al.²⁵⁸ proposed the following mechanism for the Wacker process which is compatible with isotope-effects and kinetic data obtained by other workers.

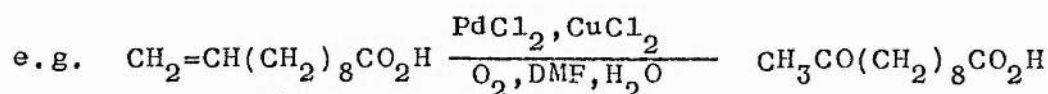


They suggested that exo-attack of water on the neutral complex (23) takes place with reversible formation of the σ -complex (24), followed by a rate-determining trans-formation of (24) to (25). Complex (25) then undergoes rapid elimination of the palladium-containing moiety and a β -hydrogen. This elimination is thought to take place in a cis-manner²⁶¹.

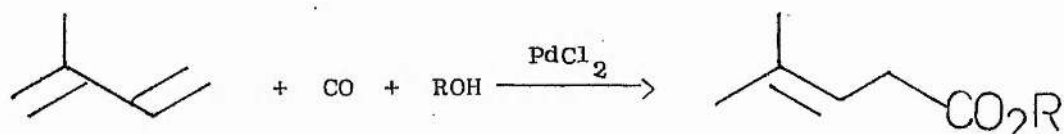
As already mentioned, ethylene as well as higher alkenes have been reacted with a variety of nucleophiles in processes analogous to the Wacker hydroxylation. When the aqueous reaction medium in the Wacker process is replaced by acetic acid terminal alkenes give vinyl acetates²⁶² while internal alkenes produce allyl acetates²⁶³. Vinyl esters are made commercially by this method²⁴⁸.



When terminal olefins other than ethylene are submitted to the Wacker reaction conditions, methyl ketones are obtained²⁴⁵, and this conversion proceed in good yield in aqueous DMF^{264,265} or sulphone²⁶⁶.

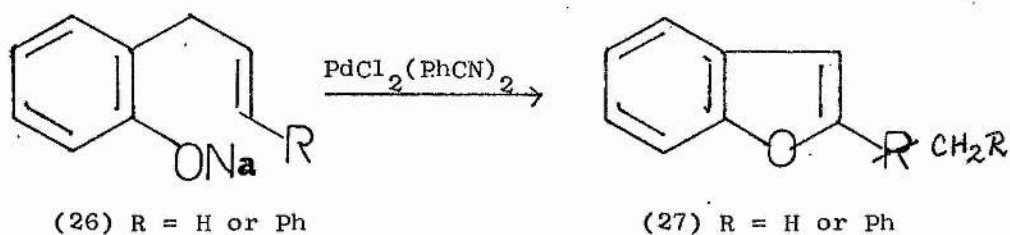


Two other reactions which are closely related to the Wacker process are the carbonylation of olefin^{267,268}

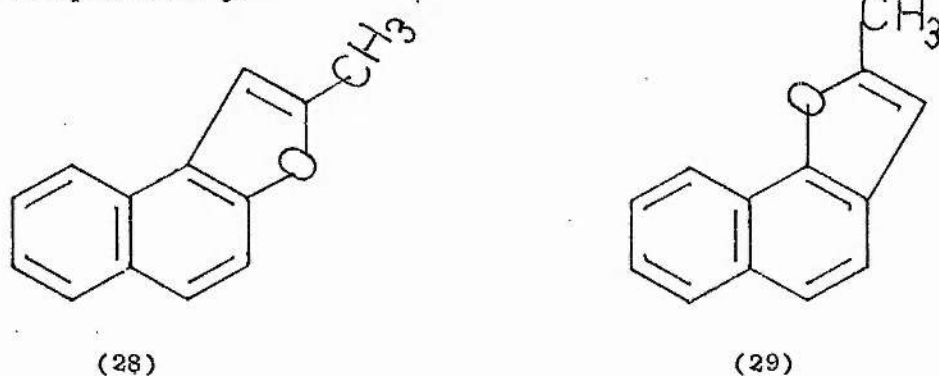


and the olefin arylation²⁶⁹, both of which are used in industry.

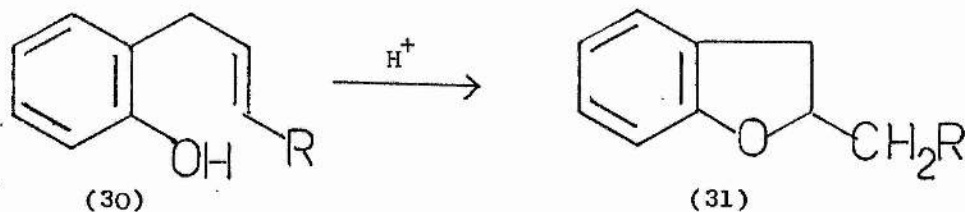
Perhaps the most important of all Pd(II)-promoted reactions is its intramolecular version in which the δ -palladium intermediate eliminates palladium hydride to effect aromatization. One of the first examples of this type of reactions was provided by Hosokawa and his coworkers²⁷⁰ in 1973, when they prepared 2-substituted benzofurans (27) by the direct cyclization of 2-allylphenols (26), in a Pd(II)-promoted reaction. This cyclization was effected



by treatment of the phenol with molar equivalents of dichlorobis(benzonitrile)palladium and sodium methoxide in refluxing benzene. Similarly, 2-methyl[2,1-b]naphthofuran (28) and 2-methyl[1,2-b]naphthofuran (29) were also prepared by reactions of 1-allyl-2-naphthol and 2-allyl-1-naphthol respectively.

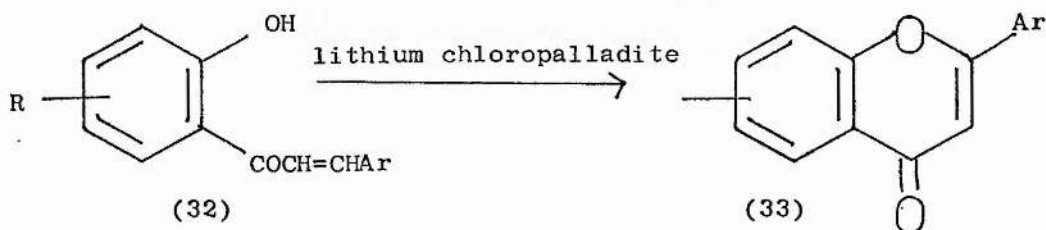


2-Allylphenols can be easily cyclized by treatment with acids²⁷¹, but the product in this case is 2-substituted dihydrobenzofuran (30). It should be emphasized that in the case of acid-catalysed cyclization, the product (31) is

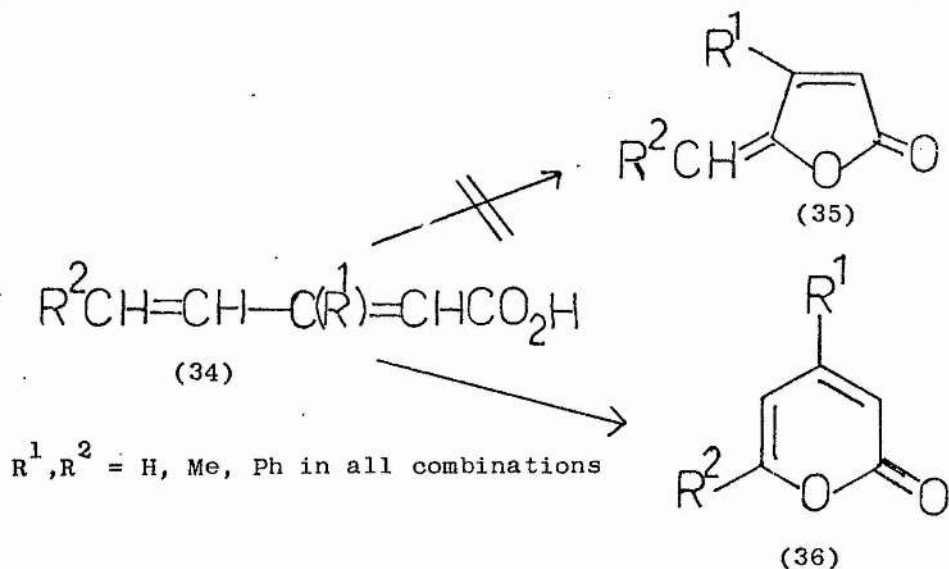


formed by the addition of the nucleophile and a proton across the double bond whereas the net result of the Pd(II)-promoted reaction is substitution at the double bond. This unique feature of Pd(II)-promoted reactions is invaluable in the synthesis of aromatic compounds.

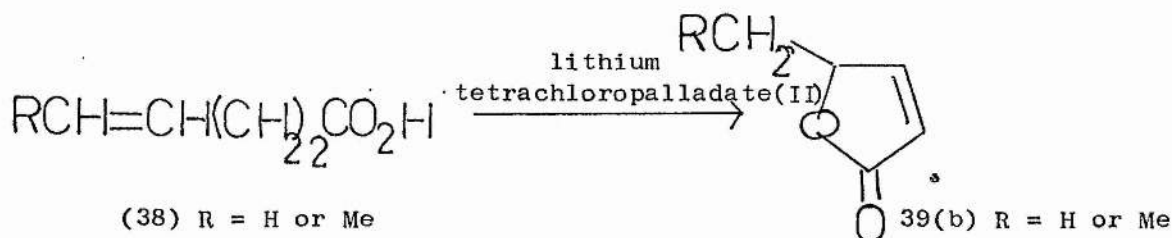
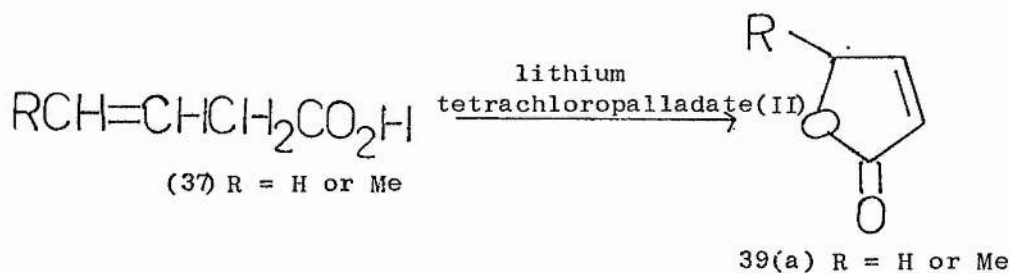
The work of Hosokawa and coworkers was followed up by Kasahara et.al.²⁷² who found that the treatment of the sodium salts of 2'-hydroxychalcones (32) with lithium chloropalladite resulted in efficient formation of flavones (33). They also reported that similar reaction of the



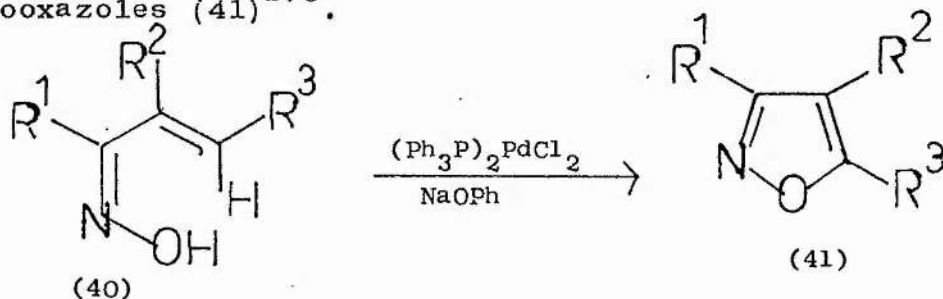
substituted penta-2,4-dienoic acids (34) gave 2-pyrones (36) and that no formation of the five-membered lactone (35) was observed²⁷³. More recently²⁷⁴, however, the same



workers showed that Pd(II)-induced intramolecular cyclization of both but-3-enoic acids (37) and pent-4-enoic acids (38) give but-2-en-4-olides (39a and 39b respectively).



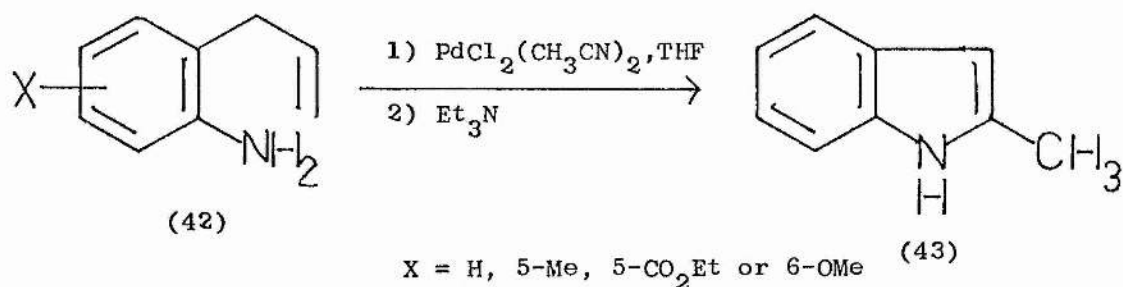
Hosokawa and coworkers who demonstrated the cyclization of 2-allylphenols into 2-substituted benzofurans, soon followed up with the finding of another facile reaction, namely, cyclization of $\alpha\beta$ -unsaturated ketoximes (40) with palladium complexes leading to the corresponding isooxazoles (41)²⁷⁵.



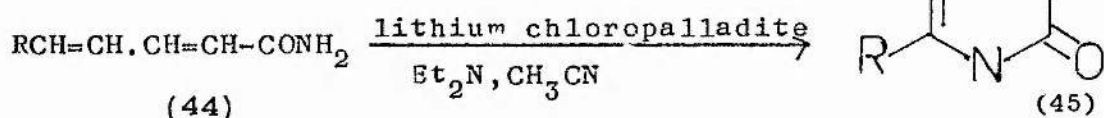
- (a) $\text{R}^1 = \text{Ph}, \text{R}^2 = \text{H}, \text{R}^3 = \text{Ph}$
 (b) $\text{R}^1 = \text{t-C}_4\text{H}_9, \text{R}^2 = \text{H}, \text{R}^3 = \text{Ph}$
 (c) $\text{R}^1 = \text{Ph}, \text{R}^2 = \text{Me}, \text{R}^3 = \text{i-C}_3\text{H}_7$

Pd(II)-assisted reactions have also been applied for both intermolecular²⁷⁶⁻²⁷⁹ and intramolecular amination

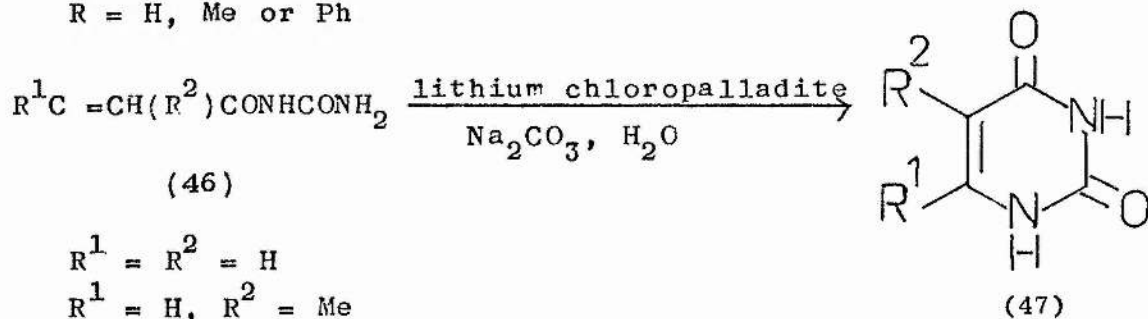
of olefins. For example, in a manner analogous to the cyclization of 2-allylphenols, 2-allyl anilines (42) have been converted to the corresponding indoles (43) via palladium intermediates²⁸⁰.



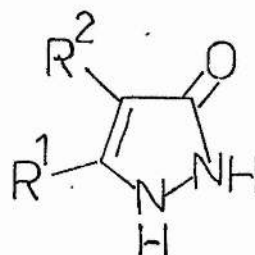
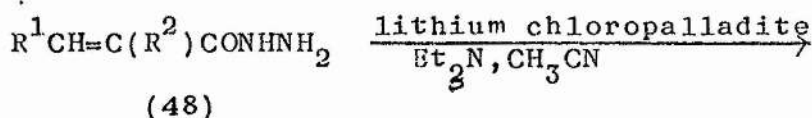
Kasahara and coworkers later used this reaction to synthesize a variety of nitrogen-heterocycles. They prepared 2-pyridones (45) from 2,4-pentadienamides (44)²⁸¹; uracil (47) from acryloylurea (46)²⁸²; and 3-pyrazolones (49) from $\alpha\beta$ -unsaturated acid hydrazide (48)²⁸³.



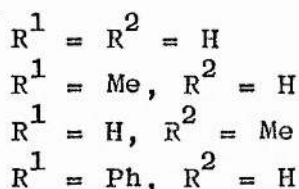
R = H, Me or Ph



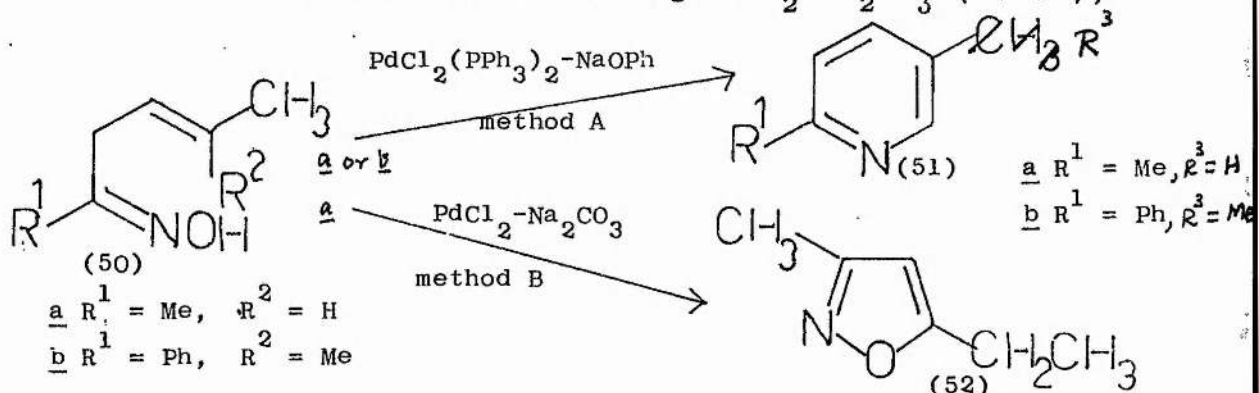
$\text{R}^1 = \text{R}^2 = \text{H}$
 $\text{R}^1 = \text{H}, \text{R}^2 = \text{Me}$
 $\text{R}^1 = \text{Me}, \text{R}^2 = \text{H}$
 $\text{R}^1 = \text{Ph}, \text{R}^2 = \text{H}$



(49)

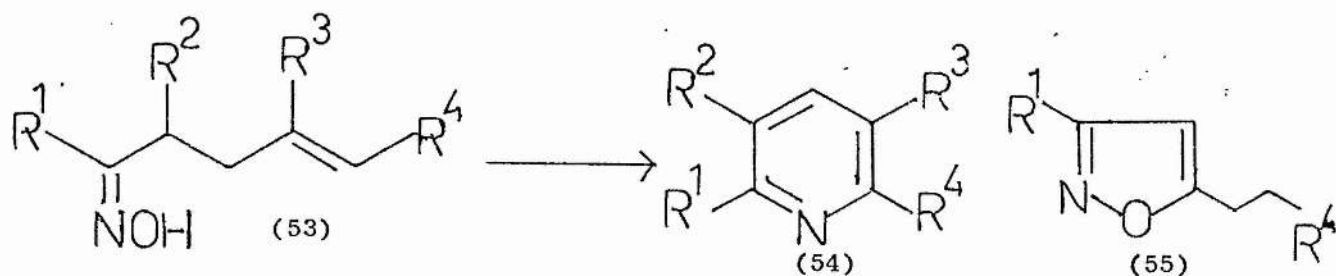


Hosokawa et.al. who earlier showed²⁷⁵ that $\alpha\beta$ -unsaturated ketoximes are transformed into isoxazoles by treatment with an equimolar amount of $PdCl_2(PPh_3)_2$ in excess NaOPh found that when this reaction is performed on $\beta\gamma$ -unsaturated ketoximes (50) pyridines (51) were obtained²⁸⁴. Interestingly, when the reaction was carried out using $PdCl_2-Na_2CO_3$ (1:1.2),



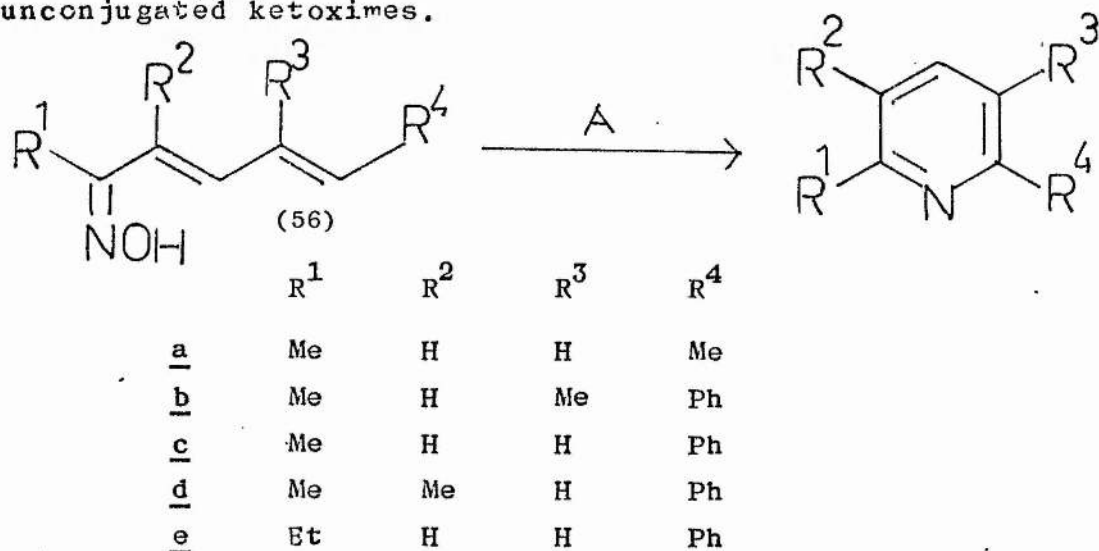
they obtained isoxazoles (52)

Analogously, under the conditions of A, γ,δ -unsaturated ketoximes 53(a-e) gave the corresponding pyridines 54(a-e) quite generally, while isoxazoles (55a) and (55d) were obtained from the oximes (53a) and (53d) by method B. The formation of isoxazoles in these reactions

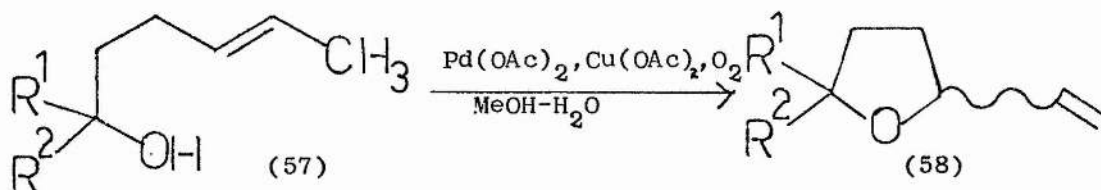


	R^1	R^2	R^3	R^4
<u>a</u>	Ph	H	H	H
<u>b</u>	Me	H	Me	H
<u>c</u>	Me	H	H	H
<u>d</u>	Ph	H	H	Me
<u>e</u>	$-(\text{CH}_2)_4-$		H	H

was explained by initial isomerization of the double bond into conjugation with the oxime group followed by intramolecular oxypalladation. The pyridine formation from γ,δ -unsaturated ketoximes 53(a-e) apparently involves the dehydrogenation process from α,β -positions in addition to coupling between the nitrogen and δ -carbon atom of the oximes. Thus, when the reaction was performed on conjugated ketoximes 56(a-e), cyclization into pyridines was affected more smoothly than in the case of aforementioned unconjugated ketoximes.



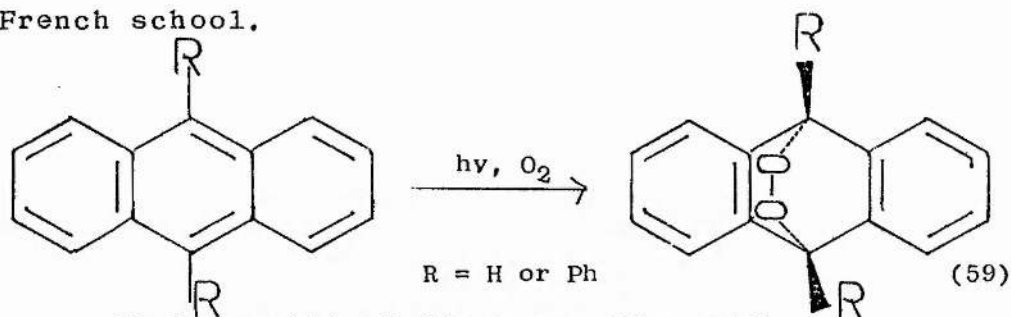
Although the classical examples of Pd(II)-promoted reactions like the Wacker process were carried out catalytically, in most of the syntheses just described a stoichiometric amount of palladium salt has been utilized. In view of the expensive nature of palladium salts, attempts have been made recently to make the cyclization catalytic with respect to the metal salt. Hosakawa et.al. achieved catalytic cyclization of 2-allylphenols into 2-substituted benzofurans by using Pd(OAc)₂ in the presence of Cu(OAc)₂ under a slow stream of oxygen²⁸⁵. They also successfully applied this catalytic system to cyclize γ,δ -unsaturated alcohols (57) into 2-vinyltetrahydrofurans (58)²⁸⁶.



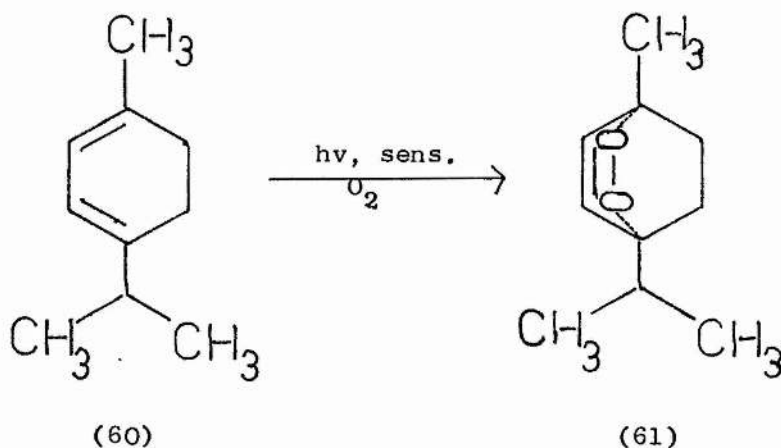
4. SYNTHESIS VIA ENDOPEROXIDES

1,4-Cycloaddition reactions of conjugated dienes with molecular oxygen to form endoperoxides have long been known²⁸⁷. In 1930 Clar described the formation of endoperoxides by irradiating pentacene in benzene solution in the presence of oxygen²⁸⁸. Such direct photochemical diene synthesis with oxygen was recognised as a general photo-reaction of the acenes by Dufraisse and collaborators²⁸⁹ in 1935, when they reported the photochemical formation of anthracene-9,10-endoperoxide (59). Since then a comprehensive

study on acene endoperoxides have been carried out by the French school.

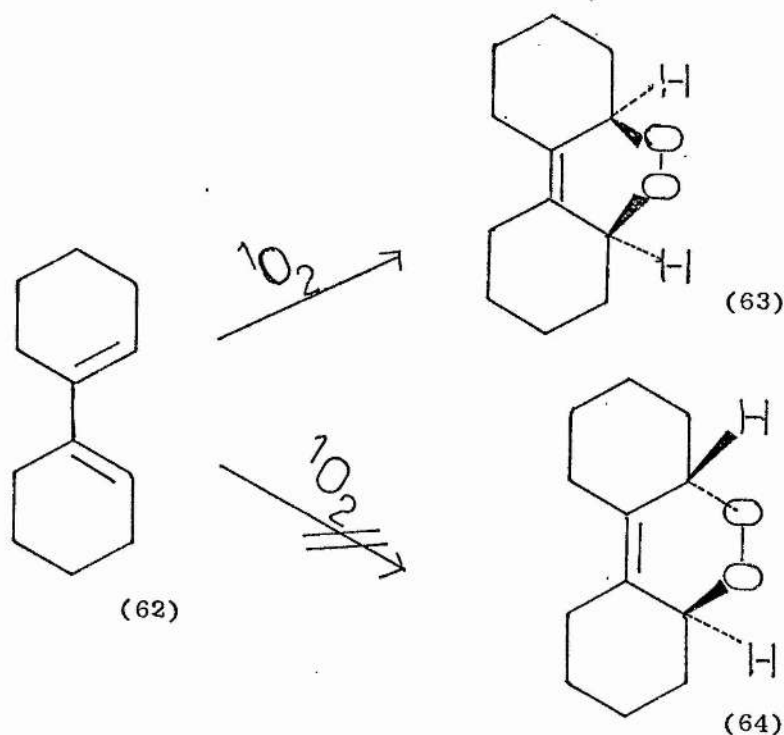


Photosensitized diene reaction with oxygen was discovered by Windaus and coworkers²⁹⁰ in 1928, when they began to investigate photochemical reactions of ergosterol and other steroidal dienes in the presence of oxygen and suitable photosensitizers (e.g. eosin) in alcoholic solutions. Early attempts by the Windaus group to extend this reaction to simpler dienes like 1,3-cyclohexadiene and α -terpinene were unsuccessful and for a long time it was assumed that this method of photosensitized 1,4-cycloaddition of oxygen was limited to steroidal systems. However, in 1941, Schenck and Ziegler²⁹¹ prepared ascaridole (61) from α -terpinene (60) by sensitized photo-oxidation and since that time many diene systems other than steroids have been found convertible to endoperoxides.



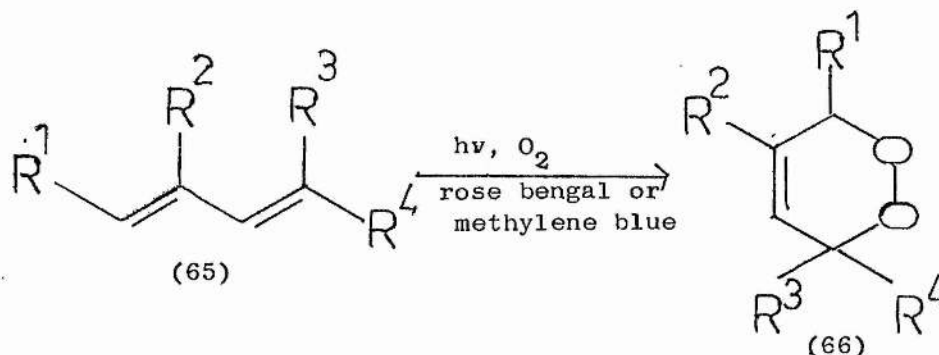
Now it is generally recognised that 1,4-cycloaddition of dienes with oxygen, whether by direct photochemical reaction or by sensitization involves singlet oxygen. In the ground state, molecular oxygen is a triplet but can be easily excited to a singlet state by interaction with sensitizers. The sensitizers usually employed are dyes such as fluorescein, its halogenated derivatives eosin and rose bengal and methylene blue. Oxygen in the excited singlet state acts as a dienophile^{292,293} and adds on to the ground state substrate in a manner analogous to the familiar Diels-Alder reaction²⁸⁷. As already mentioned polycyclic aromatic systems like anthracene can undergo direct photo-oxygenation. Kinetic studies have shown that anthracene itself acts as sensitiser for singlet oxygen formation and this, once formed, adds on to a second anthracene molecule²⁹⁴.

The concerted character of the 1,4-cycloaddition reaction of oxygen is indicated by examination of the addition of oxygen to the cisoid conformation of 1,1' bicyclohexenyl (62). The reaction leads exclusively to the cis peroxide (63). The trans product (64) could be formed in a nonconcerted two-step reaction involving formation of an intermediate diradical, but this is ruled out experimentally²⁸⁷. On the basis of these results it has been suggested that the addition of singlet oxygen proceeds through a six-membered ring transition state, analogous to the Diels-Alder reaction^{295,296}.



Most early examples of the diene reaction with oxygen contained the required cisoid butadiene structure of the oxygen acceptor as part of a carbocyclic or heterocyclic system. Until about 1972, sensitized photo-oxygenations of acyclic 1,3-dienes were extraordinarily rare in the literature and practically limited to the case of substituted buta-1,3-dienes²⁹⁷. This relative inertness of acyclic dienes towards 1,4-cycloaddition was believed to be due to the thermodynamic instability of their *s-cis* conformers. Normally, *s-trans* conformers are strongly preferred so that both the 'ene-addition' of singlet oxygen to give hydroperoxides and the formation of polymeric material would usually predominate over the 'diene-addition'.

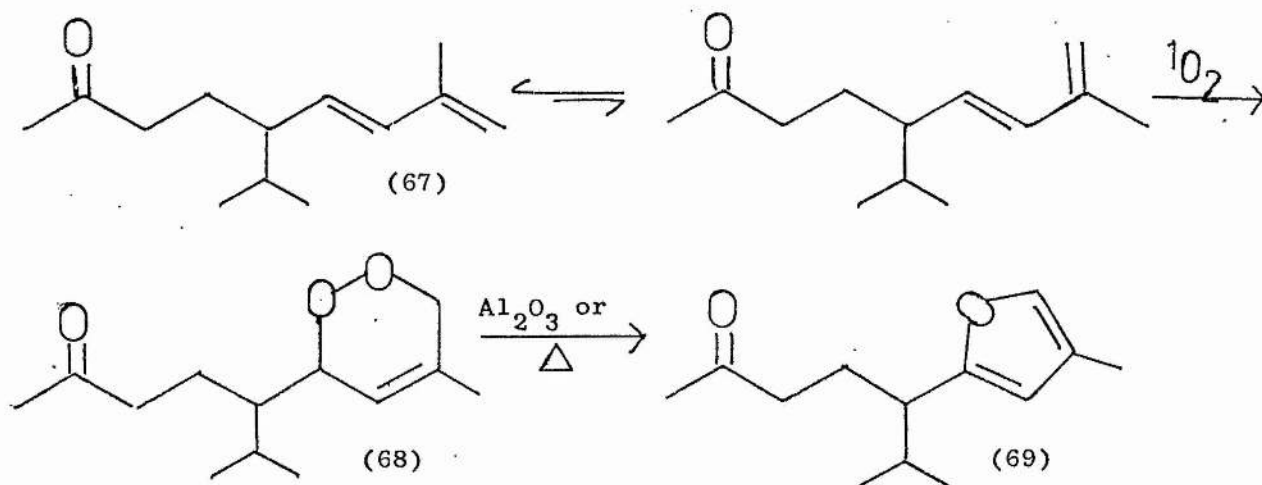
In 1972, Kondo and Matsumoto²⁹⁸ showed that isoprene and other acyclic 1,4-dienes (65) can be converted into the corresponding 1,4-endoperoxides (66) by photo-sensitized oxygenation. Since that time this reaction



- (a) $R^1 = R^3 = R^4 = H$, $R^2 = Me$
- (b) $R^1 = H$, $R^2 = R^3 = R^4 = Me$
- (c) $R^1 = H$, $R^2 = R^4 = Me$, $R^3 = Ph$
- (d) $R^1 = H$, $R^2 = Ph$, $R^3 = R^4 = Me$
- (e) $R^1 = R^4 = Me$, $R^2 = R^3 = H$

has received considerable attention particularly by terpenoid chemists who have successfully employed it as a key step in the synthesis of furano-terpenes. Preparation of furans from endoperoxides has been known from the time Rio and Berthelot²⁹⁷ obtained 2,5-diphenylfuran from trans-trans-1,4-diphenyl-1,3-butadiene via its endoperoxide.

The first application of this reaction in the terpene field was the synthesis of solanofuran (69) from solanone (67) by Berthet et.al.²⁹⁹, in 1973. The endoperoxide (68), prepared by dye-sensitized photo-oxidation of (67) was then dehydrated thermally or with basic alumina.



The generality and the powerfulness of this method has been emphasized recently by Kondo and Matsumoto who applied the reaction sequence to the syntheses of several complex naturally-occurring furanoterpenes³⁰⁰⁻³⁰². They also reported that the endoperoxide can be converted to the corresponding furan more effectively by successive treatment with t-butoxide and sulphuric acid³⁰³.

More recently Turner and Herz³⁰⁴ reported an alternative procedure for the reduction of cyclic peroxide to furan by treatment with ferrous sulphate. By this simple method they obtained excellent yields which compared well with those obtained by base-catalysed dehydration³⁰³ and were superior to those achieved by thermal dehydration²⁹⁹.

RESULTS AND DISCUSSION

I. SYNTHESIS INVOLVING PALLADIUM (II)-CATALYSIS

1. Reaction Conditions

The reaction medium was generally DMF because the reagents PdCl_2 and CuCl_2 were both readily soluble in this solvent. Methanol gave complex reaction products, presumably due to participation in the reaction.

Most reactions were performed at 90° . In general, reaction at room temperature was slow while heating above 90° lowered the yield.

The reactions were catalytic with respect to PdCl_2 . Use of stoichiometric quantities of PdCl_2 had no apparent advantage over the catalytic reaction and made the recovery of the reaction product more difficult. Passage of a gentle stream of air in the presence of CuCl_2 was adequate to reoxidize spent catalyst.

Although dry DMF and dehydrated CuCl_2 were used, the ordinary reagents were just as good.

Optimum reaction time was greatly dependent on the substrate. For instance, the reaction of methyl trans-11,12-epoxyoctadec-9-ynoate was instantaneous, and took place in the cold. Methyl vernolate, on the other hand, required over 18 h for complete reaction.

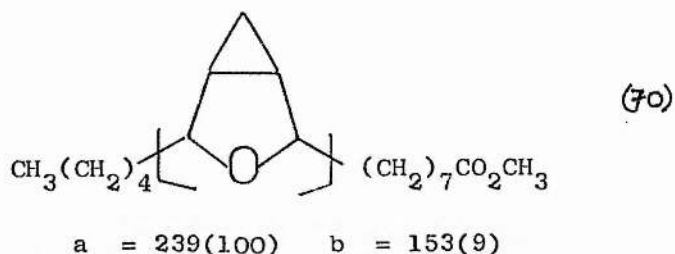
2. Methyl cis-12,13-epoxyoctadec-cis-9-enoate

Initial experiments at 60° showed that the epoxy alkene (ECL 23.7) was converted smoothly to a substance of ECL 21.2, but the reaction was slow, requiring over 3 days for completion. At 90°, complete conversion was achieved in 18 h. Though further increase in temperature speeded up the reaction, recovery of the product (ECL 21.2) was low.

The reaction product (90°) was separated into fractions A (52%), B (12%), and C (36%). On the basis of its chromatographic (GLC, TLC) and spectroscopic (UV, IR, PMR, and MS) behaviour, fraction A was identified as the 10,13-furan. There was no evidence in the mass spectrum of A for any significant quantities of the 9,12-isomer. The 10,13-compound is one of the furanoid products of acid-catalysed reaction of methyl linoleate-diepoxides²¹⁷, but the pure compound has not been prepared before.

Fraction B contained three main components of ECL 21.6, 24.9, and 25.8. They were not separable from one another by TLC and moved in a single band, which was slightly less polar than the starting epoxy ester. The mass spectrum of the component of ECL 21.6 (obtained by GC-MS) showed the molecular ion peak at 310 and the base peak at m/e 239. It also contained a peak at m/e 153 and on the mass spectral evidence and also on the basis of its polarity, we believe that the compound of ECL 21.6 is the bicyclic ether (70). This substance has been previously recognized as one of the minor rearrangement products of

methyl vernolate with boron trifluoride^{305,306}.



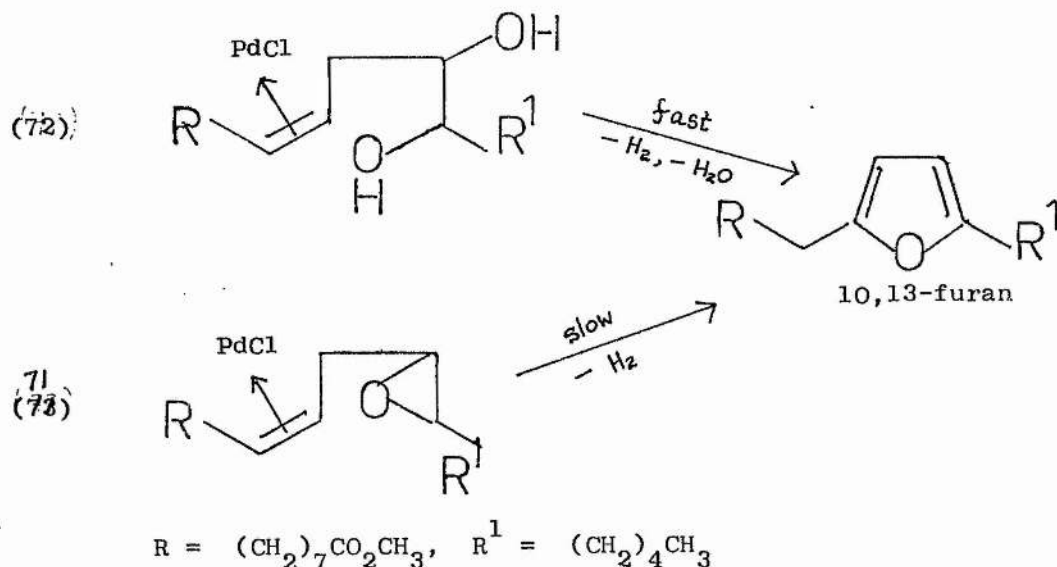
The major products of BF_3 -catalysed rearrangement of methyl vernolate are 12- and 13-oxooleates³⁰⁵⁻³⁰⁶. Conacher and Gunstone³⁰⁶ found that these keto esters are accompanied by cyclopropane compounds, which were mainly cis and trans isomers of methyl 9,10-methylene-12-oxo-hepta-decanoate, and also by small amounts of the bicyclic ether (70). Their cyclopropane esters had ECL values of 24.8 and 25.6 respectively, and like our fraction B they migrated with keto esters on TLC. We did not examine the last two components of B (ECL 24.9 and 25.8), but on the basis of their GLC and TLC properties, they may be the same cyclopropyl esters found by Conacher and Gunstone.

Fraction C was an admixture of polar material. They probably consisted of hydroxy and keto compounds but we did not characterize them.

3. Methyl threo-12,13-dihydroxyoctadec-cis-9-enoate

This reaction is considerably faster than the corresponding one with methyl vernolate and more than 80% of the starting material reacted within 20 min. The only reaction product was the 10,13-furan, which expectedly is the same compound as that obtained in the previous reaction (identification is by chromatographic and spectroscopic

behaviour, see Experimental Section for details).



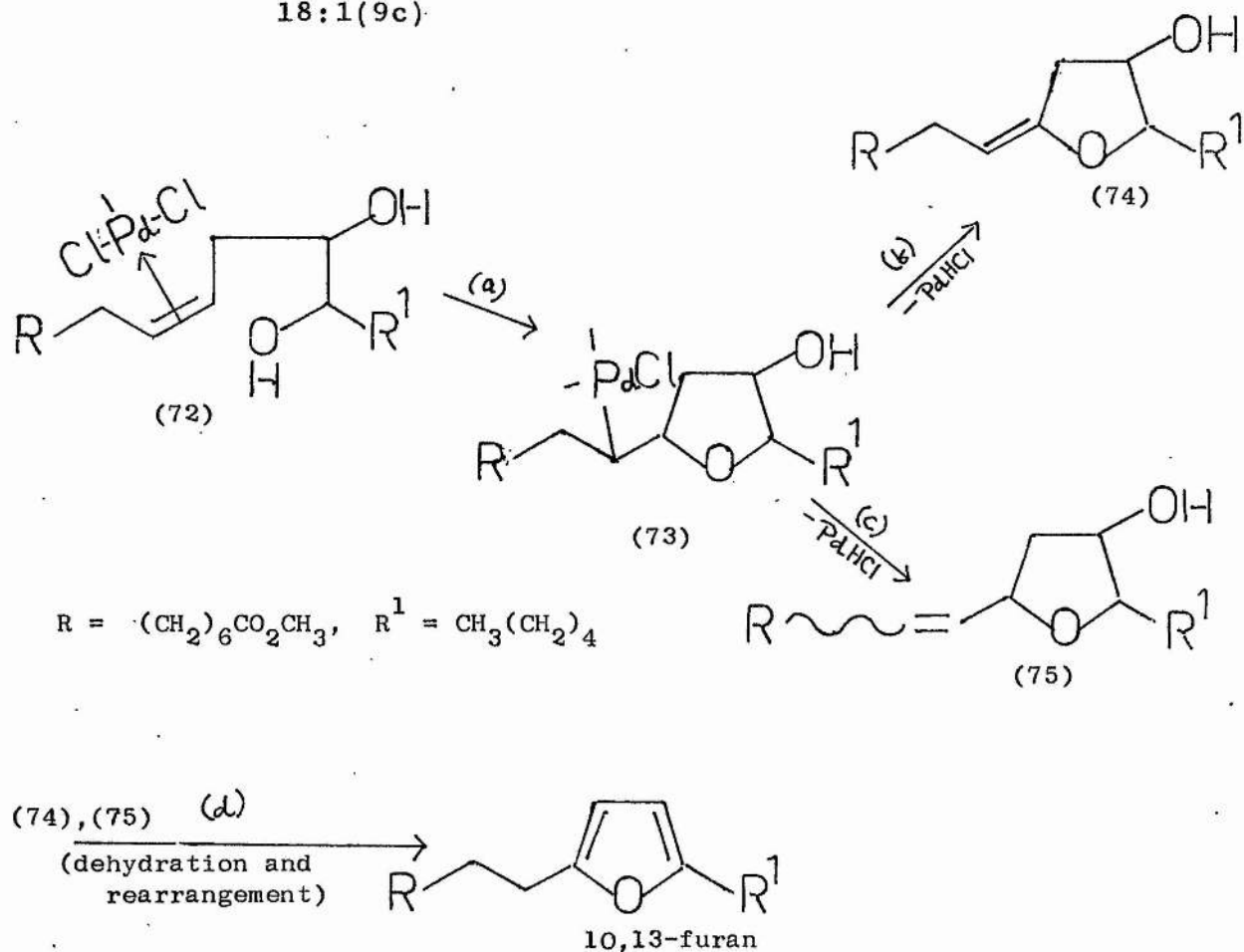
The dihydroxy ester (72) is related to the epoxide (71) and in fact it was prepared from the latter. The faster reaction rate of the former over the latter may be due to the greater facility of the former in attaining the transition-state geometry (shown in the diagrams) required for ring closure. Because of the rapid reaction rate and the absence of by-products, the route using the dihydroxy ester is preferred for the preparation of the 10,13-furan.

The dihydroxy alkene (72) contains both β - and γ -hydroxyl groups with respect to the double bond and the absence of products due to involvement of the β -hydroxyl (9,12-furan, in this case) is noteworthy. The regiospecific formation of 10,13-furan shows that the reaction of the γ -hydroxyl group is dominant. Differences in behaviour between β - and γ -hydroxy cis alkenes has been observed before. For example, THF esters were readily formed during

oxymercuration-demercuration³⁰⁷ and halogenation³⁰⁸ of methyl 9-hydroxyoctadec-cis-12-enoate (a γ -hydroxy alkene); but no cyclic products were obtained in the corresponding reactions with methyl ricinoleate (a β -hydroxy alkene). Reasons for this apparent inability of β -hydroxy alkenes to cyclise will be presented later.

The requirement for a second hydroxyl group for the furanization is shown by the fact that, when submitted to Pd(II)-promoted reaction the γ -hydroxy alkene methyl 9-OH, 18:1(12C), does not give furans [see Section II (4)]. We suggest the following mechanism^{an} for the reaction.

Scheme:4 Formation of 10,13-furan from methyl 12,13-di OH 18:1(9c)



Intramolecular cyclization of (72) involving the γ -hydroxyl group and the double bond activated by π -coordination to Pd(II) gives the THF (73), in which the palladium is σ -bonded to C(9). This is followed by hydride abstraction from either C(10) or C(8) to give vinyl ether (74) or the allyl ether (75). We have no evidence for the formation of the allyl ether and it appears that hydride abstraction of the ring takes precedence over abstraction from the chain. Dehydration of (74) and (75) gives the corresponding diunsaturated ethers which then undergo rearrangement to the thermodynamically more stable 10,13-furan.

Ring closure involving the γ -hydroxyl group and C(9), followed by steps (b)-(d), should lead to a pyran. We have no evidence for such, but in view of the labile nature of pyrans, even if they were formed in the reaction they might not have survived the reaction conditions (air).

4. Methyl 9-hydroxyoctadec-cis-12-enoate

In the light of the results obtained in the previous reaction, we were interested to know the outcome of the reaction of 9-OH 18:1(12c) [a γ -hydroxy alkene] with Pd(II). In reactions promoted by Pd(II), the double bond is regenerated after ring closure, and we expected an unsaturated THF ester to be formed as the main product.

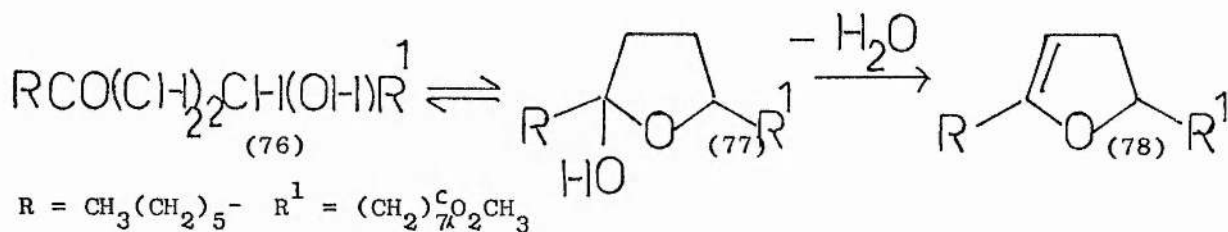
(i) Investigation of reaction time

The reaction was carried out in the usual way. GLC of some preliminary experiments showed that the reduction of the peak at ECL 24.9 due to starting material was accompanied by the appearance of several new peaks of which that of ECL 21.3-21.4 was largest. After reaction at room temperature, overnight, there was 73% starting material and 16% of product of ECL 21.3. After reaction for one further hour at 90° there was no starting material and the major GLC peak had an ECL 21.3. After one further hour this peak was also very small and a series of experiments showed that the maximum yield of the material of ECL 21.3 was after a reaction period of only 20 min at 90° (see Table 31 for full GLC details of this product).

(ii) Investigation of reaction products

Attempts to isolate a pure product proved difficult. The major component on TLC has a R_f value of 0.08 and appeared just behind the starting material (R_f 0.10). Rather surprisingly for such a polar product, its GLC showed a major peak of ECL 21.3. Rechromatography gave a fraction with GLC peaks at 21.3 (90%) and 20.5 (10%). Other TLC fractions of higher R_f value also gave this GLC peak at 21.3, and we suspected that some or all of the products are unstable on GLC and decomposed under GLC conditions to the product of ECL 21.3.

A fraction similar to the product (R_f 0.08) isolated by TLC was obtained by crystallization of the reaction product from petrol at -20° . Its GLC showed the peak of ECL 21.3 only. The IR spectrum of this material showed absorptions for hydroxyl (3340 cm^{-1}) and for two different carbonyl groups (1710 and 1740 cm^{-1}) and it was considered that it may be the ketol (76) which decomposed in the GLC to give an unsaturated cyclic ether possibly of the structure shown (78) and by the reaction sequence indicated:



Any 9-hydroxy-13-oxo ester might produce a similar dihydropyran derivative. Our conclusion that the major product of the reaction is a ketol is further evidenced by the fact that the peak of ECL 21.4 was replaced by one at 24.7 after silylation. The mass spectrum of the silylated derivative showed that the original compound is methyl 9-hydroxy-12-oxooctadecanoate. The cyclisation reactions of such ketols to dihydrofurans on silicone columns, at 180° , have been reported³⁰⁹.

As a parallel investigation the total reaction product was subject to (a) catalytic hydrogenation, (b) chemical reduction, and (c) chemical reduction followed by hydrogenation. In each case the reaction product was examined by GLC before and after silylation

Table 31: GLC^a of the reaction product of methyl 9-OH 18:1(12c) with Pd(II), and its various derivatives

<u>P^b</u>	<u>P(TMS)^c</u>	<u>R(H)</u>	<u>R(H)(TMS)</u>	<u>R(BH₄)</u>	<u>R(BH₄)(TMS)</u>	<u>R(BH₄)(H)</u>	<u>R(BH₄)(H)(TMS)</u>
18.5(5)	15.0(3)	18.1(9)	17.9(8)	16.0(9)	16.0(3)	16.0(6)	16.2(2)
				18.1(9)	18.0(3)	18.0(45)	18.0(7)
	18.4(3)			18.3(32)	18.4(10)		
		19.6(7)	19.5(6)			19.4(2)	
	20.4(16)		20.2(16)		20.4(6)		20.1(5)
		20.7(56)	20.6(55)			20.6(22)	20.5(3)
21.5(59)	21.3(20)				21.4(3)		
	21.8(10)		21.8(7)		21.8(7)		21.8(6)
				22.6(11)	22.5(63)		22.0(4)
							22.5(71)
24.2(3)	23.6(3)	23.8(4)	23.6(4)		23.5(3)	23.6(3)	
24.7(3)	24.7(45)	24.6(17)	24.7(2)	24.6(11)		24.5(16)	
				24.9(11)			
25.0(17)							
25.4(5)	25.4(5)			25.3(11)		25.3(6)	
				25.7(3)			
				26.2(3)			

a The first figure gives the ECL (DEGS) and the figure in parenthesis gives the percentage area - small peaks (area <2%) are not reported

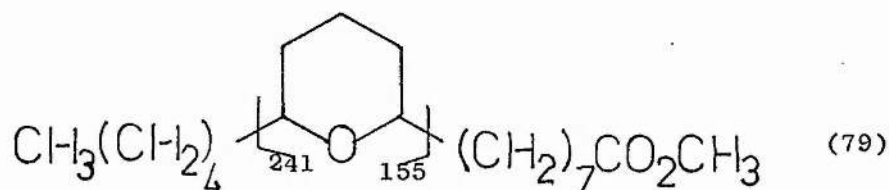
b P refers to the total reaction product

c The letters in parenthesis refer to the operations carried out on the reaction product, in that order. TMS, H, and BH₄ stand for silylation, hydrogenation, and sodium borohydride reduction respectively.

and full results are detailed in Table 31. The nature of the major derivatives are discussed in the following sections.

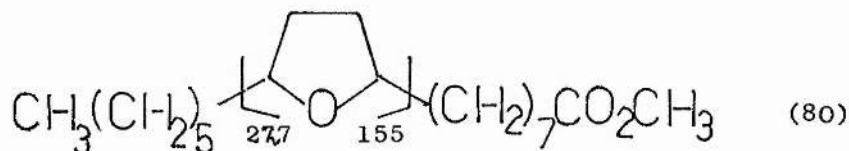
(a) Catalytic hydrogenation

When submitted to hydrogenation in the presence of palladized charcoal, the product from the previous reaction (P) gave a material which showed a double peak of ECL 20.7 and 20.9 as the major component. Prep. TLC of this material furnished four fractions: A (10%), B (10%), C (51%), and D (29%). Chromatographic and mass spectral evidence showed that A is methyl stearate, which was probably formed from hydrogenolysis of unreacted starting material. The mass spectrum of B had the molecular ion peak at m/e 312, which indicated that B is an oxygen-containing C_{18} ester. Its low ECL (19.5) and R_f (0.81) suggested that B is a cyclic ether. The base peak at m/e 155 and a peak at m/e 241 showed that this compound is a tetrahydropyran ester of the structure shown (79). This assignment was confirmed by the presence



of peaks due to sequential loss of water and methanol from the ion at m/e 241; and also by peaks due to loss of water from the ion fragment at m/e 155³¹⁰.

Fraction C was identified as methyl 9,12-epoxyoctadecanoate (80). Silicic acid TLC using PE30 as the developing solvent readily separated this compound (R_f 0.75) from the isomeric THP ester. C showed a double peak (ECL 20.7 and 20.9) on GLC, which was presumably due to cis and trans isomers. Its mass spectrum showed the molecular ion at m/e 312 and the base peak at m/e 155 and another peak at m/e 227 confirmed its structure. As in the case of the THP ester, this structural assignment was supported by presence of ions due to loss of water and methanol from the m/e 227 ion and due to loss of water from the m/e 155 fragment.



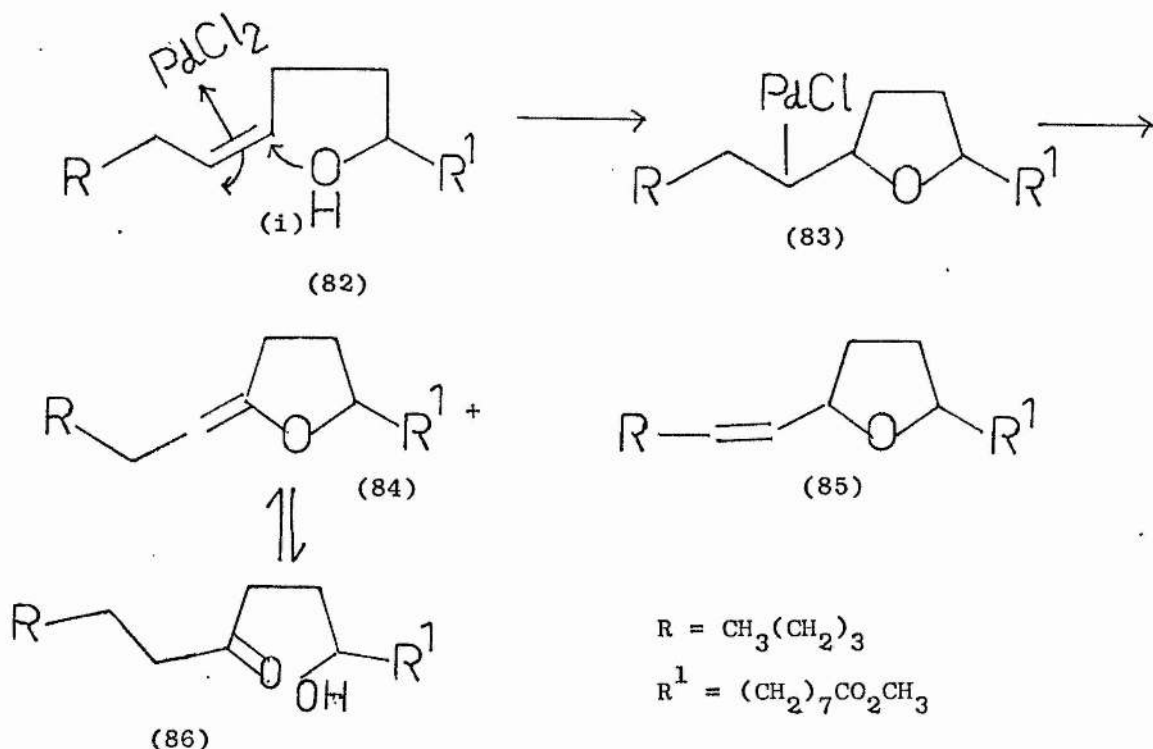
Fraction D was identified as methyl 9-hydroxystearate by its mass spectrum. This compound was probably formed by hydrogenation of unreacted starting material.

(b) Chemical reduction

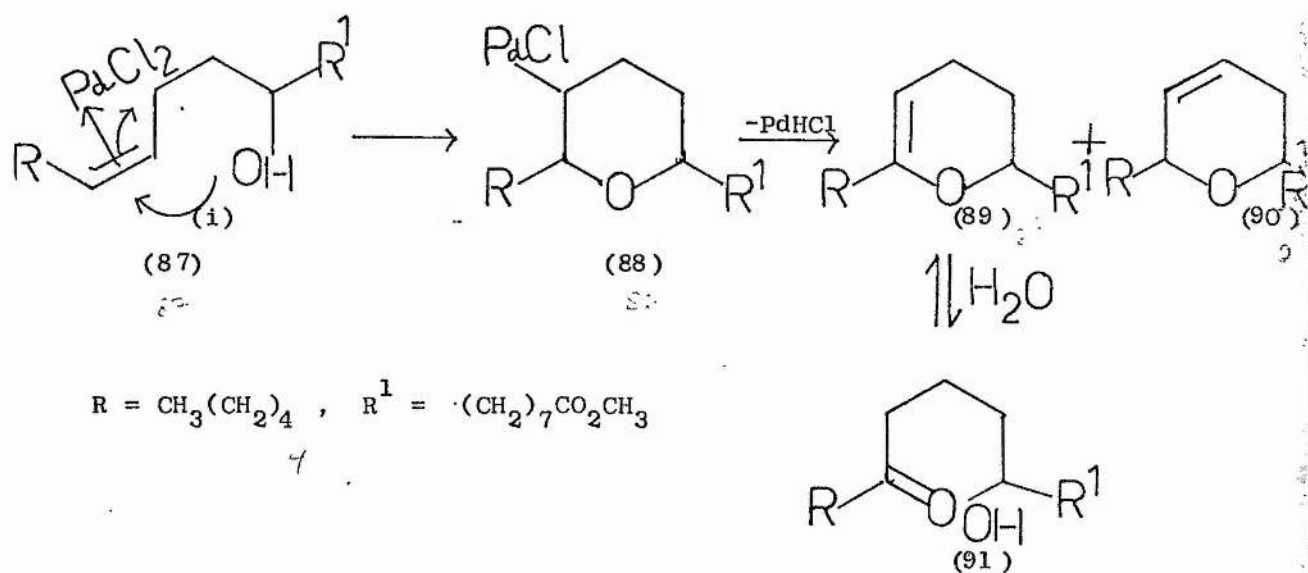
After reduction of P with sodium borohydride, the major GLC peak at ECL 21.4 disappeared and GLC response of the product was low. Silylation of this reduction product produced a new peak at ECL 22.5 of magnitude corresponding to the original peak at ECL 21.4 (Table 31). A similar result was obtained with the crystallized reaction product. The silyl ether had the ECL expected of a bis-OTMS derivative of a dihydroxystearate and its mass

(iii) Explanation of the Reaction

Pd(II)-catalysed cyclization of (82) gives the δ -Pd intermediate (83) which has two possibilities for β -elimination of a palladium hydride: one is from the ring C-12, leading to the vinyl ether (84); and the other is from C-14, to give (85). GLC properties of (85) are expected to be similar to that of (84) and some of the less polar material in the total reaction product may be due to (85). Since the major product was (86), which we consider to arise from the labile vinyl ether (84), it follows that (84) is formed regioselectively in the β -hydrogen elimination step. This supports the views expressed earlier that elimination of a hydrogen attached to a ring takes precedence over one in a chain.



The presence of the tetrahydropyran ester (80), among the hydrogenation products of P shows that the δ -ketol 9-OH, 13-oxo 18:0 is also formed in the original Pd(II)-promoted reaction. This δ -ketol probably arises from initial ring-closure involving the 9-OH and C(13) followed by β -elimination of a palladium hydride to give ethers of type (89) and (90). The former isomer



then undergoes hydrolysis to give the δ -ketol (91). We show later that the cyclization of (87) to give the 6-membered ring (88) is a favoured process.

(iv) Attempt to Prepare Methyl 12-hydroxy-9(10)-oxooctadecanoate

To substantiate our conclusions that the main product of the present reaction is a γ -ketol, we attempted to prepare a similar compound by an independent method. Hg(II)-catalysed hydration of the triple bond in methyl 12-hydroxyoctadec-9-ynoate is expected to give a mixture of 12-hydroxy-9-oxo and 12-hydroxy-10-oxo stearates. GLC of

the reaction product showed a single peak of ECL 21.1, which was probably due to the decomposition of the γ -ketol in the GLC column. Any β -ketol was not expected to undergo such change and because of its high polarity it was not seen on GLC prior to silylation. Silylation gave a major peak of ECL 24.7, which is the same value as that of the silylation of product of the γ -ketol obtained in the Pd(II) catalysed reaction, thus supporting our structural assignment for the reaction product, P.

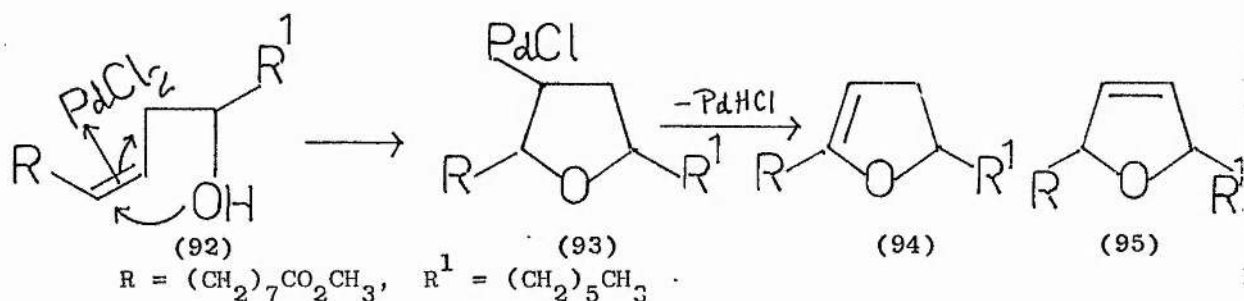
5. Methyl 12-hydroxyoctadec-cis-9-enoate

The ease with which the γ -hydroxy alkene reacted with Pd(II) in the previous reaction prompted us to investigate the reactivity of methyl ricinoleate (a β -hydroxy alkene) under the same conditions. This was tried in spite of the fact that cyclization of β -hydroxy alkenes to produce 5-membered oxygen heterocycles is regarded as a disfavoured process, (this subject is discussed later).

Performed in the usual way, the reaction was much slower than that with the γ -hydroxy alkene, requiring 10 h for completion. GLC of the reaction product showed two overlapping peaks of ECL 20.8 and 20.9 as the major components, which were readily separated by TLC into fractions A (16%) and B (61%) respectively. Two other polar fractions C (9%) and D (14%) were also isolated.

Compound A was identified as 9,12-furan by its chromatographic and spectroscopic behaviour. B contained

mainly the component of ECL 20.8. GLC examination of the hydrogenation product of B showed a double peak of ECL 20.5 and 20.8 in the approximate ratio of 3:1. This hydrogenated material was identified as methyl 9,12-epoxystearate by its mass spectrum and also by comparison with an authentic sample. The IR spectrum of B lacked bands for hydroxyl or keto functions and therefore B is not a ketol, unlike the main product of the previous reaction. A dihydrofuran ester is a conceivable product of this reaction and B had the polarity expected of such a compound. Its PMR spectrum, which contained a multiplet at 4.64 (protons adjacent to ring oxygen) and a singlet at 5.70 (olefinic protons) provided evidence for a dihydrofuran structure. Consideration of the usual mechanism of Pd(II)-



promoted cyclizations shows that two dihydrofuran derivatives, namely, (94) and (95) are possible. If one isomer were to form exclusively, then that compound should be easily distinguished by its PMR spectrum. For example, if (95) is the only product it will have PMR signals for two olefinic protons and two protons next to ring oxygen, whereas, if (94) is the sole product its PMR spectrum will contain one-proton signals for each kind. The spectrum of B had approximately one-proton integrals for each kind

and therefore (94) is the main, if not the only product. The mass spectrum of B showed the molecular ion at 310, the base peak at m/e 153, and another intense peak at m/e 225. These ions confirm the 9,12-dihydrofuran structure without indicating the double bond position. Catalytic hydrogenation of B produced 9,12-THF ester.

The successful cyclization of methyl ricinoleate (92) to the 5-membered ring (93) has important mechanistic implications. In this connection it is expedient to summarize a set of the rules for ring closure put forward by Baldwin^{312,313}. The following nomenclature is used. A ring-forming process is described with the prefix Exo, when the breaking bond is exocyclic to the smallest so formed ring and Endo correspondingly. A numerical prefix describes the ring size, and the suffixes Trig (trigonal) and Dig (digonal) indicate the geometry of the carbon atoms undergoing ring-closure reaction.

The Rules are as follows:

Rule 1: Trigonal Systems

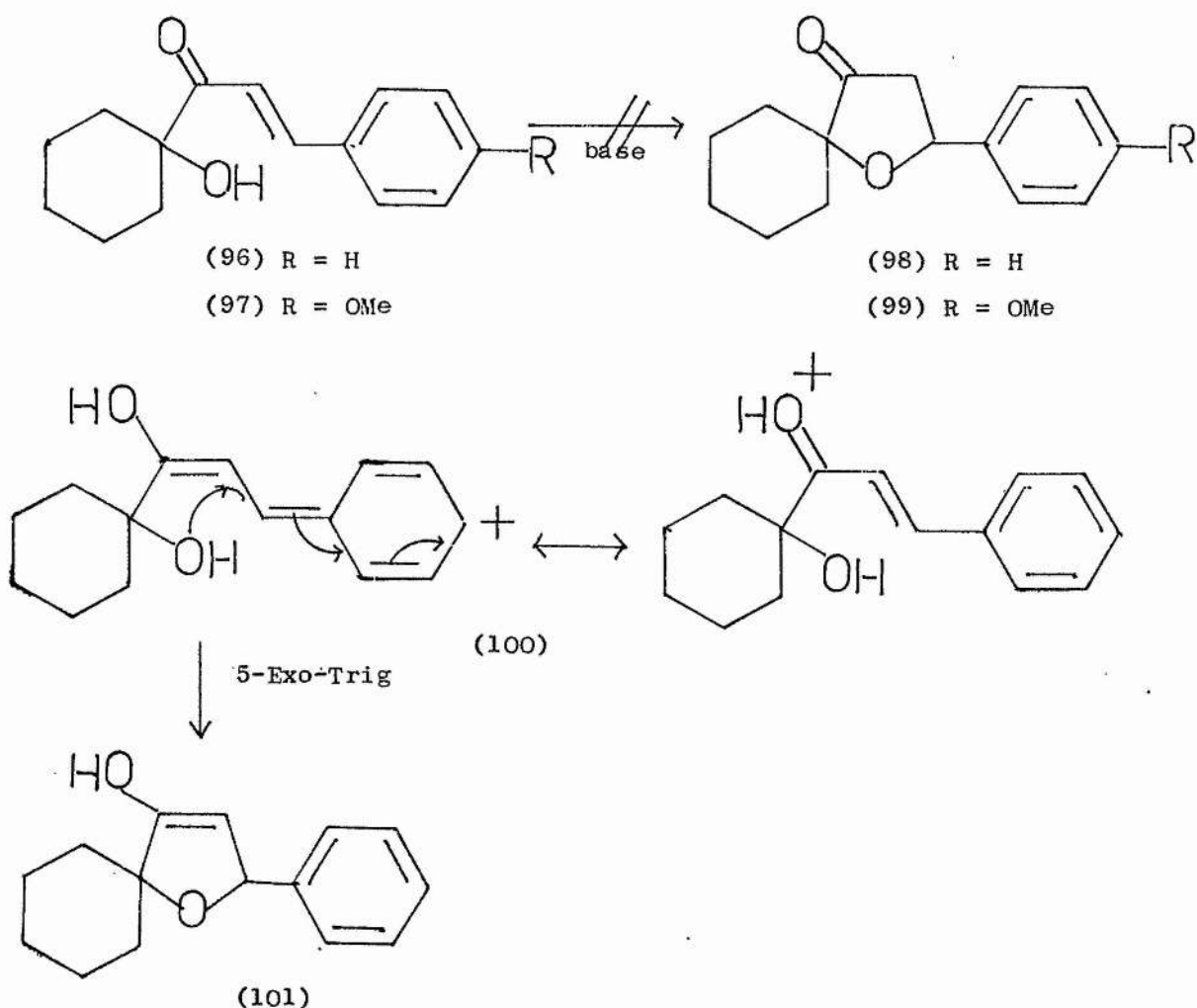
- (a) 3 to 7-Exo-Trig are all favoured processes.
- (b) 3 to 5-Endo-Trig are disfavoured; 6 to 7-Endo-Trig are favoured.

Rule 2: Digonal Systems

- (a) 3 to 4-Exo-Dig are disfavoured processes; 5 to 7-Exo-Dig are favoured.
- (b) 3 to 7-Endo-Dig are favoured.

To form a tetrahydrofuran ring, methyl-ricinoleate has to cyclize by a 5-Endo-Trig process. It has been found that for first-row elements this is a disfavoured process, alternative reactions of the type 5-Exo-Trig taking precedence³¹⁴. Just one case of a 5-Endo-Trig ring closure for a second-row element, sulphur, has been found³¹⁴; no case of a facile 5-Endo-Trig closure for oxygen has been hitherto reported.

Baldwin *et.al.*³¹⁴ failed to cyclize the β -hydroxy ketols (96) and (97) to the furanones (98) and (99) under a variety of basic conditions (through a 5-Endo-Trig process). However, on acid catalysis (96) and (97) were efficiently



closed to the ketones (98) and (99) respectively. They explained this successful cyclization as a result of contributions from structures of type (100) which permit the favoured 5-Exo-Trig mode of closure.

For methyl ricinoleate to form a 5-membered cyclic ether by such a 5-Exo-Trig process the double bond has to be between C(8) and C(9). Although double bond migrations are possible in Pd(II)-promoted reactions²⁴⁶, such a situation is unlikely in the present case because the isomer so formed is a γ -hydroxy alkene, which from the results of the previous experiment does not yield cyclic end products. Though the reaction is slow, methyl ricinoleate forms stable cyclic products suggesting that the resistance to ring closure is a result of a kinetic rather than a thermodynamic barrier. Indeed, Baldwin et.al.³¹⁴ found evidence for such and suggested the existence of a steric barrier for this type of cyclization. They also noted that, sulphur, in some circumstances facilitates the normally disfavoured 5-Endo-Trig process, and suggested that this may be a general phenomenon for second-row elements since their larger radii and bond lengths allow them to obtain conformations which are difficult for the corresponding first-row elements.

Formation of furan as a by-product in this reaction is surprising and we suggest that prolonged exposure of methyl ricinoleate to CuCl_2 results in the formation of the corresponding vinyl chlorides which undergo dehydrochlorination to give 12-OH 18:1(9a). We show below that

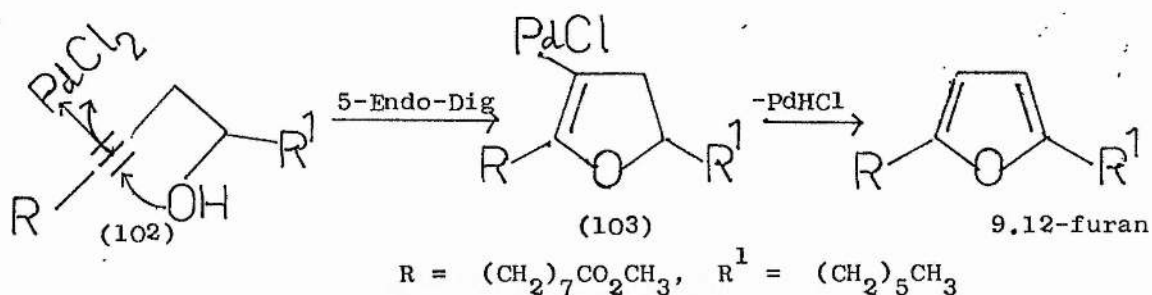
this hydroxy alkyne readily cyclizes with Pd(II) to form 9,12-furan.

6. Methyl 12-hydroxyoctadec-9-ynoate

Two modes of ring closure are possible for this compound, namely, 4-Exo-Dig and 5-Endo-Dig. According to the Baldwin Rules³¹², only the latter process is favoured and therefore a 5-membered ring was expected as the sole product. This substrate was of special interest because the anticipated product was a furan.

The reaction was carried out in the usual way and all of the starting material had reacted after 20 min. GLC examination of the product showed two overlapping peaks of ECL 20.7 and 21.1 as the major components and also peaks at ECL 17.7 (11%) and 23.3 (10%). Prep. TLC readily separated the product of ECL 20.7 (fraction A, 61%) from the others (fraction B, 39%).

Chromatographic and spectroscopic examination proved that A is 9,12-furan. Furans have been previously made by cyclization of γ -hydroxy alkynes but the presence of a cis double bond between the hydroxy and alkyne groups was required¹⁷⁷. In spite of numerous studies on Pd(II)-promoted nucleophilic attack on alkenes, similar reaction with alkynes have not been investigated. We postulate the following mechanism for this reaction.



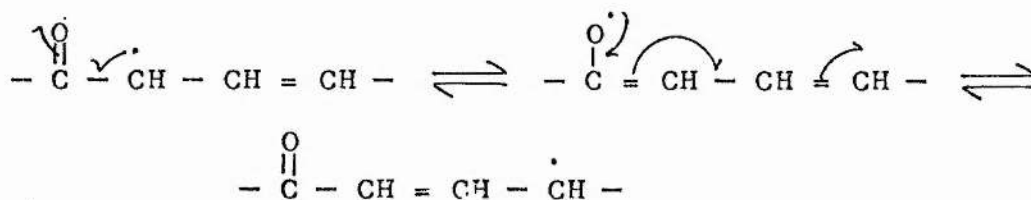
GLC of fraction B showed a peak of ECL 20.9 as the major component. On TLC it had polarity similar to that of hydroxy-containing esters and an ECL as low as 20.9 was unexpected for such a polar compound. We suspected that this is a γ -ketol like the product obtained with 9-OH 18:1(12c), and this view was supported by IR evidence which showed the presence of hydroxyl (3460 cm^{-1}) and keto (1710 cm^{-1}) groups. Final confirmation came from hydrogenation, when methyl 9,12-epoxystearate was obtained.

Formation of carbonyl compounds from alkynes, via hydration catalysed by Hg(II) is well known. It is not surprising therefore, that Pd(II) can promote such hydration to yield ketones. Although anhydrous reaction conditions were used initially, formation of water during the regeneration of catalyst is inevitable (See Introduction).

7. Methyl 12-oxooctadec-cis-9-enoate

This keto-alkene resembles methyl 12-hydroxy-octadec-9-ynoate in that it contains an oxygen function at C-12 and has overall unsaturation of two units. Since the latter compound furnished a furan by Pd(II)-promoted reaction, we expected a similar result with the keto-alkene.

Formation of a 5-membered oxygen heterocycle from the keto-alkene, methyl 12-oxooleate is possible only by a 5-^{Endo}~~exo~~-Trig ring closure, which according to Baldwin Rules and also in light of the sluggish reactivity of methyl ricinoleate, is a kinetically disfavoured process. We therefore, expected this keto-alkene to react slowly and yield furan. On the contrary, all of the starting material reacted within 20 min and no furans were obtained. TLC showed only polar material and GLC did not show any peaks before or after silylation. We have noted that methyl 12-oxooleate does not keep well, even when stored in petrol, and we believe that this is due to autoxidation. This keto-alkene is particularly susceptible to auto-



(104)

oxidation because of the enhanced stability of the free radicals (104) and bubbling of air through it, as done in the present case, may accelerate the oxidation.

8. Methyl 12-hydroxyoctadec-trans-9-enoate

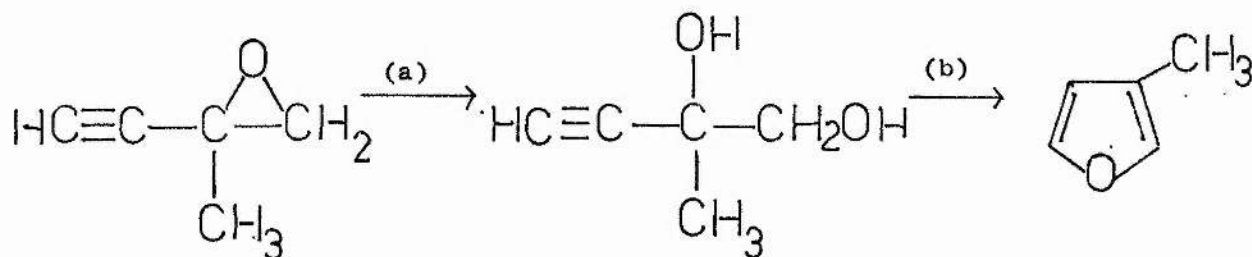
As in the previous reaction, all of the starting material reacted within 20 min to give polar products. Effect of the geometry of the double bond on the rate or the course of the Pd(II)-promoted reaction has not been studied. We have no plausible explanation for the rapid formation of polar material with the trans isomer.

9. Methyl *trans*-11,12-epoxyoctadec-9-ynoate

Pd(II)-promoted ring closure normally increases the overall unsaturation of the reaction product, as in the conversion of methyl vernolate (an epoxy alkene) to 10,13-furan. Therefore, the epoxy alkyne used in this experiment was expected to yield an unsaturated furan.

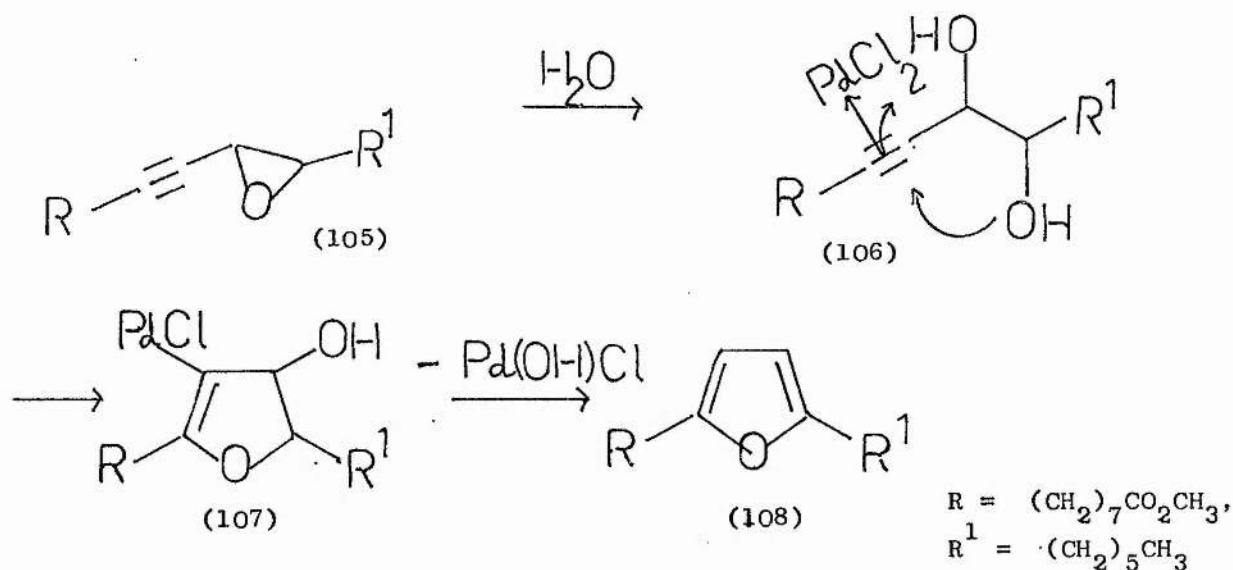
Palladium powder deposited upon mixing the reagents in the cold indicating that this is a fast reaction. GLC and TLC showed that all of the starting material had reacted by that time. The product was a single compound (BCL 20.6) and was purified by TLC. Its PMR spectrum did not show the olefinic signals expected of it. The mass spectrum showed the molecular ion peak at 308 and was identical with that of authentic 9,12-furan. On this evidence and also by comparison of its UV, IR, and chromatographic properties with the authentic material, we concluded that the product is 9,12-furan.

Abbot and Gunstone²¹⁷ also obtained 9,12-furan by Hg(II)-catalysed rearrangement of the same epoxy alkyne. Earlier Miller²²² presented evidence that this conversion proceeds through an acetylenic diol intermediate.



(a) 2 M H₂SO₄; (b) HgSO₄, H₂SO₄

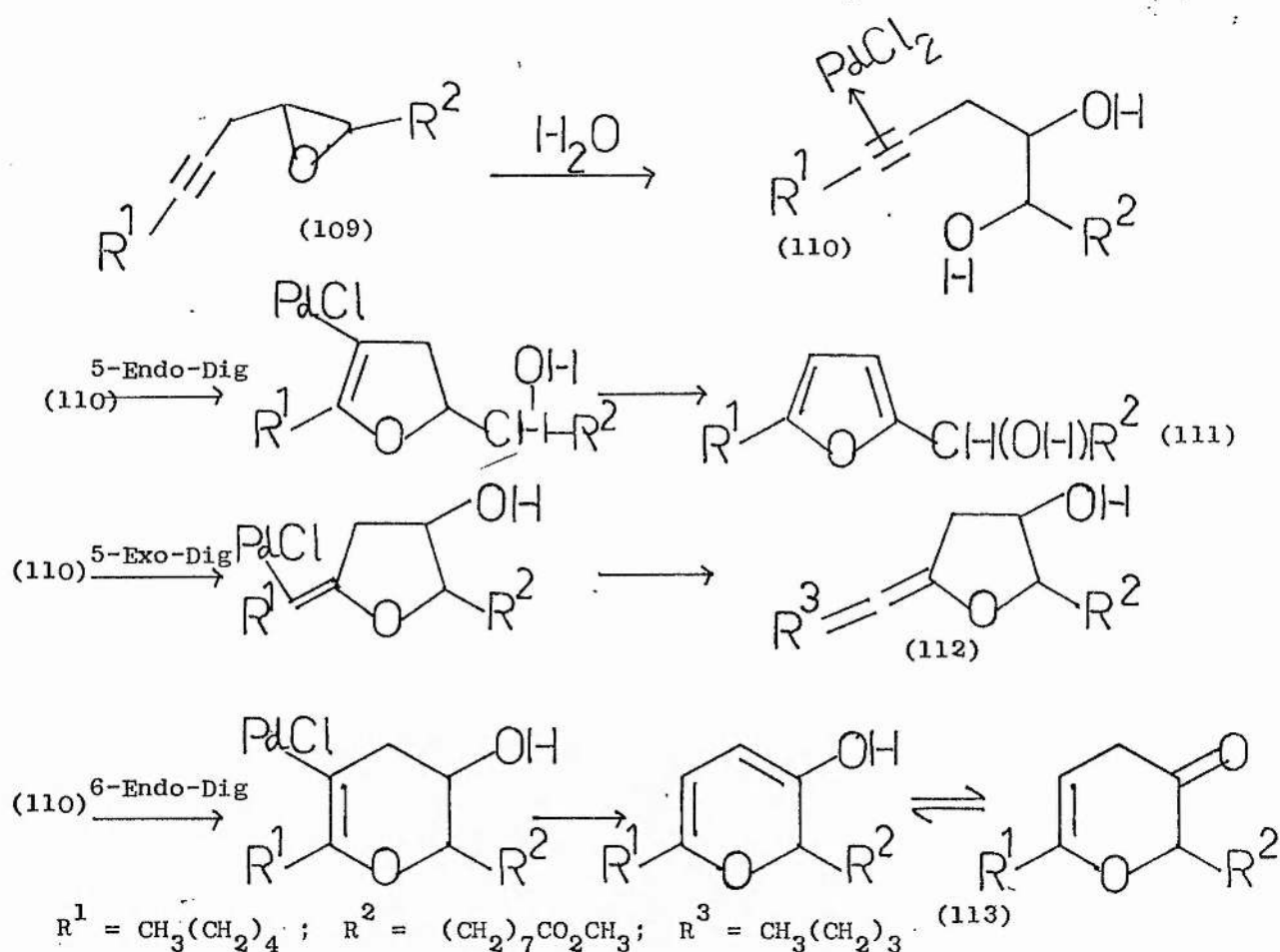
It is conceivable that in the Pd(II)-promoted reaction too the epoxide (105) is ring opened to diol (106) water is formed during the regeneration of catalyst, which cyclizes in the usual manner [cf 12-OH 18:1(9a)] to give the (107). Intermediate (107) has no β -hydrogen for



elimination and it is possible that it eliminates the hydroxyl on C-11 to furnish the furan (108). Opening of the epoxide ring prior to cyclization into a 5-membered ring may explain the failure to form an unsaturated furan.

10. Methyl *cis*-9,10-epoxyoctadec-12-ynoate

The reaction was carried out according to the usual procedure. All of the starting epoxy alkyne had reacted after 30 min. but only polar products were obtained. Assuming that epoxide ring-opening occurs as in the previous reaction, the diol so formed (ii) can react in a variety of ways. The intermediate (110) is both a β - and γ -hydroxy alkyne and cyclization involving the β -OH may lead to the



hydroxy furan (111). Ring closure involving the γ -OH can occur by either a 5-Exo-Dig or a 6-Endo-Dig process (of which the former is favoured³¹⁵) to give the allene (112) and the pyranone (113) respectively. We do not believe that the hydroxy furan (111) was formed, because this substance should be visible on GLC. Compounds (112) and (113) might have been present among the polar products but these were too complex and we did not attempt to characterize them.

Whilst these considerations help to explain the complexity of the reaction product, it should also be noted that alkynes undergo polymerization in the presence

of Pd(II). The principal product with acetylene itself is trans-polyacetylene, and di-substituted acetylenes give cyclobutadienes²⁴⁸. The recovered yield of furan in the reaction of trans-11,12-epoxy 18:1(9a) with Pd(II) was only 57%, though it was the sole product of the reaction. This reduced yield may also be due to polymerization of the starting material catalysed by Pd(II).

II SYNTHESSES FROM 1,2-EPOXIDES

1. Introduction

Preparation of furans by cyclo-dehydration of γ -diket^{con}ones has long been known, and Abbot et.al. obtained 9,12-furan by boron trifluoride-catalysed cyclization of methyl 9,12-dioxostearate²¹⁶. It is also well-known that boron trifluoride-etherate promotes rearrangement of epoxides to ketones, but Maerker et.al.³¹⁶ observed that the yield of diketones expected from the reaction with methyl linoleate-diepoxydes was poor. Later Abbot and Gunstone showed that this reduced yield of diketones was due in part to dehydration of the γ -diketones so produced into furans.

An alternative procedure for the rearrangement of epoxides to ketones using propyl iodide, sodium iodide, and DMSO was described by Kenner et.al.²¹⁹. Lie Ken Jie and Lam, attempting to use this method to prepare diketones from the diepoxydes of methyl linoleate, obtained a mixture of 9,12- and 10,13-furans instead²²⁰. For a re-examination

these findings we carried out the reaction on the diepoxides of methyl linoleate and linolenate, and report below our results together with the surprising discovery that methyl vernolate (an epoxy alkene) forms 9,12-furan under the same reaction conditions.

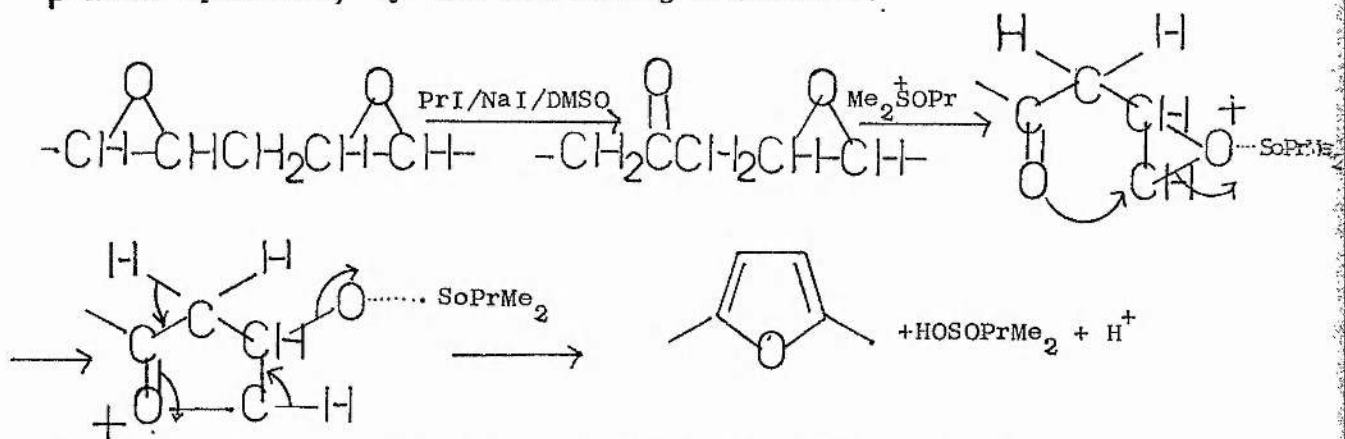
2. Methyl cis-9,10-cis-12,13-diepoxyoctadecanoate

The reaction product was separated into three fractions: A (42%), B (17%), and C (41%). A was a mixture of 9,12- and 10,13-furans and on the basis of its mass spectrum A contained these two isomers in approximately equal amounts.

Fraction C showed strong oxo carbonyl absorption (1710 cm^{-1}), and it had the same polarity as that of authentic methyl 9,12-dioxostearate. The mass spectrum showed the molecular ion peak at 326 and contained significant peaks at m/e 270, 256, 199, 185, 184, 170, 155, 141, 127, 114, 113 and 99. On this basis C contains both 10,12- and 9,13-dioxo stearate, but some of these fragment ions can arise from the 9,12- and 10,13-isomers and the complete absence of the latter compounds cannot be claimed. The PMR spectrum contained a sharp singlet at 2.54 ($-\text{COCH}_2\text{CO}-$) which we take as evidence for the presence of the β -diketone

The isolation of these diketones contradict the report by Lie Ken Jie et.al.²²⁰ that no dioxostearate was formed in this reaction. They used a considerably longer reaction time (5 h) and although cyclization of the 10,12- and 9,13-diketones into 4- and 6-membered cyclic ethers is conceivable such products have not been hitherto reported.

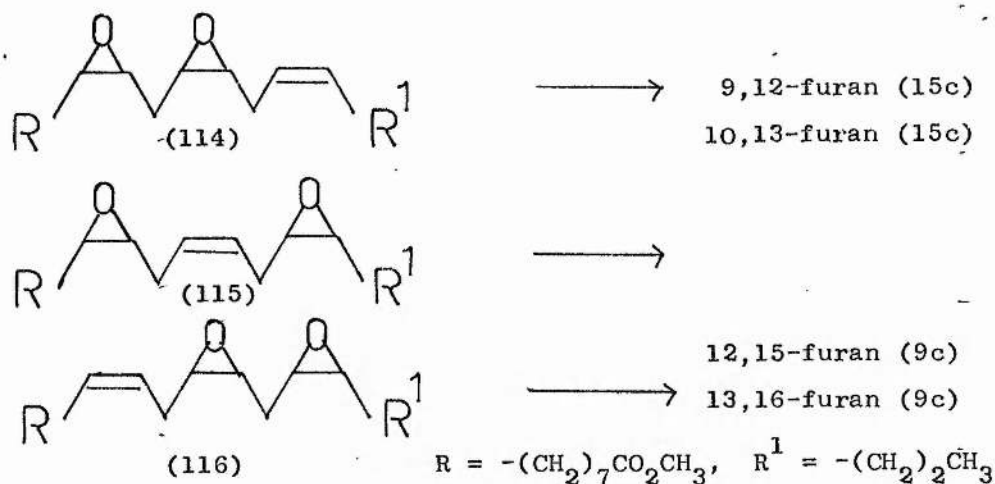
They also claimed that the formation of furans in this reaction does not occur by dehydration of γ -diketone intermediates but by cyclization of the corresponding β -keto epoxides, by the following mechanism.



Support for this reaction mechanism came from the fact that, when methyl cis-9,10-epoxy-12-oxostearate is treated with PrI-DMSO , 9,12-furan is obtained. Replacement of the reagent with BF_3 or *p*-toluenesulphonic acid gave the same product. This conversion has been effected more efficiently by HCl -catalysis²²¹. A furanization reaction involving a β -keto epoxide with *p*-toluenesulphonic acid was reported by Fietel and Baranger³¹⁷.

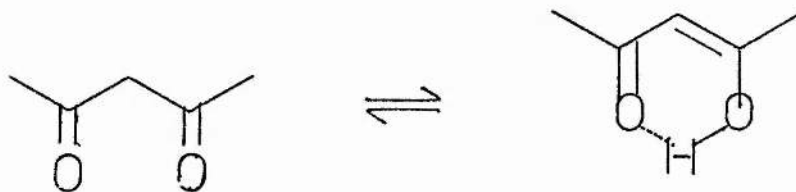
3. Diepoxides of methyl octadeca-cis-9, cis-12, cis-15-trienoate

A mixture of the three isomeric diepoxides (114)-(116) were prepared by controlled epoxidation of methyl linolenate. Since the diepoxides (114) and (116) each contain one double bond, the anticipated products were the monounsaturated furans indicated below.



Furans were not expected from the diepoxide (115) because the two oxirane rings are separated by four carbon atoms. The reaction product was separated into fractions A (31%), B (36%), and C (33%). GLC examination of A showed three main peaks and their ECL values were that expected of monounsaturated C_{18} F esters. Polarity of A (silica acid TLC) was similar to that of authentic 9,12-furan. The IR spectrum of A showed all the absorptions characteristic of long-chain furans and an additional peak at 3000 cm^{-1} (vinyl C-H) indicated olefinic functions. Evidence for olefinic groups was also found in the PMR spectrum which contained a complex signal at 5.3-5.5 in addition to the furanoid proton signal at 5.74. Final confirmation came from the mass spectrum which showed the molecular ion peak at 306. The olefinic bonds are not conjugated with the furan ring, because the UV absorption maximum for A was at 222 nm which is the same as that observed for the saturated furans. On this evidence and also by consideration of the integral ratios of the various proton kinds in the furanoid products, we believe that the double bonds are retained in the original positions, i.e. C(9)-C(10) and C(15)-C(16).

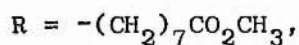
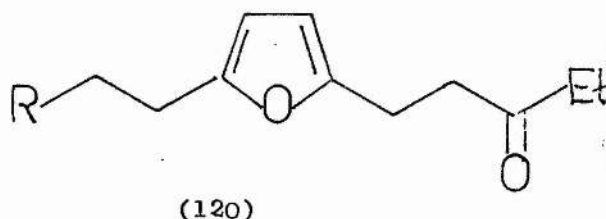
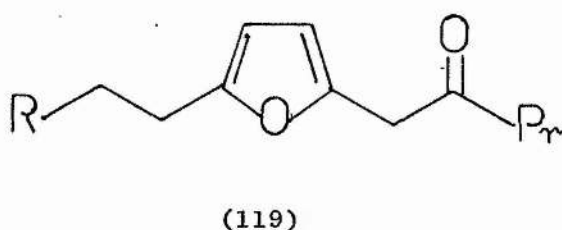
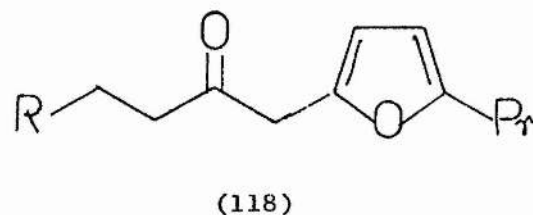
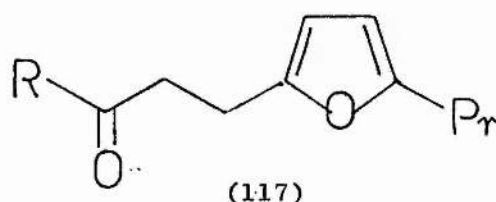
Fraction C showed intense oxo carbonyl absorptions (1720, 1680 and 1580 cm^{-1}) and this material had the same polarity as authentic methyl 9,12-dioxostearate. The IR peaks at 1680 and 1580 suggest the presence of β -diketones, the lower C=O stretching frequencies been probably due to enolization:



Presence of hydrogen bonded hydroxy groups was indicated by a peak at 3480 cm^{-1} . GLC of fraction C showed three peaks with ECL values (29.0, 29.6, and 31.0) comparable with that of methyl 9,12-dioxostearate (29.0). The PMR spectrum contained signals (2.14-2.56) due to protons of type $-\text{CH}_2\text{CO}-$, but due to the complexity of this signal structural assignment could not be made on its basis. The mass spectrum showed the molecular ion peak at 324, which is in agreement with mono-olefinic diketone structure and the ion fragments indicated several diketones 13,15- and 12,16-($\Delta 9$); 9,15- and 10,15-, 9,16-, and 10,16-($\Delta 12$); 10,12- and 9,13-($\Delta 15$). The 12,15- and 13,16-($\Delta 9$); and 9,12- and 10,13-($\Delta 15$), which are expected to cyclodehydrate to form furans, have ions common with the other dioxo isomers, so the absence of these compounds in fraction C cannot be confirmed.

Fraction B proved to be most interesting. Its IR spectrum showed all the absorptions typical of long-chain furans, yet on TLC, it was well-separated from the furans

in Fraction A. Polarity of B was similar to that of authentic methyl 12-oxooleate and we suspected B is a keto furan, which was supported by strong oxo carbonyl absorption (1720 cm^{-1}). The PMR spectrum showed signals due to furanoid protons (5.76) but no olefinic protons were indicated. The mass spectrum showed the molecular ion at 322 and consideration of the various fragment ions revealed that fraction C contained a mixture of four isomeric keto furans: 9- and 10-oxo 12,15-furan; and 15- and 16-oxo 10,13-furan.



The formation of these keto furans has interesting mechanistic manifestations. Their formation must clearly involve interaction between the residual epoxy and olefinic functions. The 12,15-pair of isomers can then be derived from either (114) or (115), and the 10,13-pair from (115)

or (116). We investigated the reaction of methyl vernolate with sodium iodide-propyl iodide-DMSO and report below that a furan could indeed result from the interaction of the epoxide with the double bond and that this reaction is regiospecific.

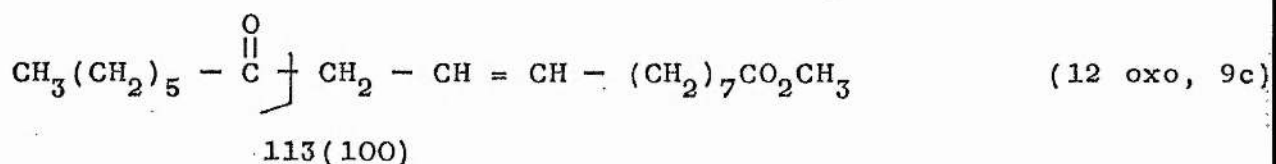
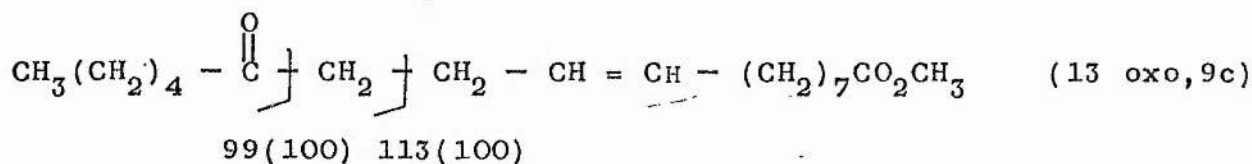
4. Methyl cis-12,13-epoxyoctadec-cis-9-enoate

The reaction product was separated into fractions A (37%), B (38%), C (12%), and D (13%). Fraction A was identified as 9,12-furan, on the basis of its chromatographic and spectroscopic behaviour. This ready conversion of the epoxy alkene to a furan confirms our view that the keto furans obtained in the previous reaction resulted by reactions involving the olefinic functions. Since the reaction conditions used were those which rearrange epoxides to ketones, it is possible that the ring closure to furan involves interaction between the resultant keto functions with the olefinic groups. We later show that cyclization of β -keto alkenes to furans did indeed occur under the present reaction conditions (see following reaction).

The regiospecific formation of the 9,12-furan is surprising. The Pd(II)-promoted reaction of methyl vernolate gave the 10,13-isomer only, and here is an example of a reaction where exclusive formation of different isomers is obtained from the same starting material by use of different reagents. In the light of the regiospecificity of the reaction, the absence of keto furans expected from the methyl linolenate diepoxide (114) and (116), namely, 13,16-

9,12-keto furans respectively, indicates that the keto furans (117)-(120) arise only from the diepoxide (115)

Fraction B showed strong oxo carbonyl absorption (1720 cm^{-1}). It had polarity similar to that of authentic methyl 12-oxooleate and its ECL was the same as that of the latter although the peak was broader indicating more than one isomer. The PMR spectrum of B contained mainly the signals expected of methyl 13-oxooleate but resonances attributable to the protons of the 12-oxo isomer, for example, 3.0 ($-\text{COCH}_2\text{CH}=\text{CH}-$) were also present. The mass spectrum of B showed the molecular ion at 310. Presence of the 13-oxo isomer is confirmed by the mass peak at m/e 99. An ion at 113 is also base peak and although this indicates occurrence of a comparable proportion of the 12-oxo isomer too, an alternative explanation for this ion is possible. β -cleavage in oxo compounds has been reported³¹⁸ and this



fragmentation is particularly favoured in 13-oxooleate because its β -position is also allylic. This problem was resolved by examining the mass spectrum of the hydrogenation product of B, which now contained the two α -cleavage ions 99 and 113 in approximately 3:1 ratio, showing that B is

predominantly methyl 13-oxooleate. The latter ion can still be due to β -cleavage of the 13-oxo isomer but the presence of a McLafferty rearrangement ion at m/e 128, which can arise only from the 12-oxo isomer is evidence for small quantities of the latter. Fractions C and D were not examined.

5. Methyl 12-oxooleate ~~+ β -hydroxy alkene~~

Purification of the reaction product afforded a material (19%), which was identified as 9,12-furan. The poor yield may be attributed to the lability of the starting material, as noted previously. Our attempts to cyclize methyl ricinoleate (β -hydroxy alkene) ^{were} ~~was~~ unsuccessful and it follows that the presence of keto or epoxy functions is necessary for the reaction.

We are not able to give a plausible mechanism for the cyclization of methyl vernolate or methyl 12-oxooleate with PrI-DMSO . Though ring closure involving an olefinic bond coordinated to sulphur may be envisaged, formation of a 5-membered ring from 12-oxooleate has to occur by a 5-Endo-Trig mode which is kinetically unfavoured³¹³. The isomerization of epoxides to ketones is also realized in nonsulphurous solvents such as DMF, methyl ethyl ketones and acetonitrile³¹⁹, and involvement of sulphur in the furanization reaction could be checked by carrying out the reaction in such a medium.

III SYNTHESSES VIA 1,4-ENDOPEROXIDES

1. Methyl octadeca-trans-8,trans-10-dienoate

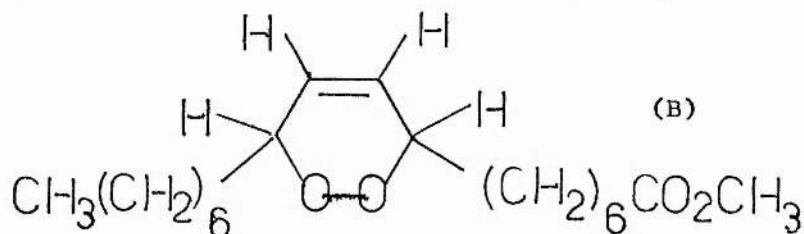
(a) Sensitized Photooxidation

Despite popular use of rose bengal³⁰⁰⁻³⁰² as sensitizer in photooxidation of acyclic 1,3-dienes, we preferred to use methylene blue because the reaction with the latter was quicker. Rose bengal faded rapidly during the reaction and required to be replaced often. The progress of oxidation was monitored by TLC, which showed the appearance of a product more polar than the starting diene. GLC was not suitable because the ECL values of the starting material and product were similar.

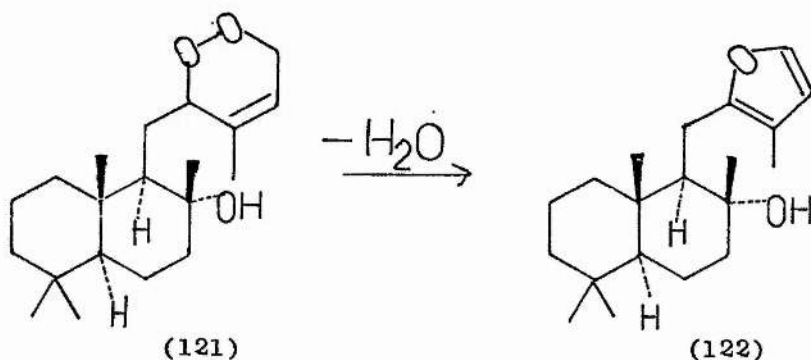
After 5 days of reaction, the product was separated into fractions A (¹⁴~~42~~%), B (74%), and C (12%). Fraction A was unreacted starting material. It showed strong trans-1,3-diene absorption at 990 cm^{-1} and its polarity was similar to that of the starting diene ester. GLC showed two peaks of ECL 20.3 (20%) and 21.0 (80%). The latter ECL is the same as that of the starting material and the peak at ECL 20.3 was originally present as a minor contaminant. We confirmed that A is unreacted starting material by submitting it to further photooxidation, when more of compound B was obtained.

Though their ECL values were similar (21.0 and 21.2 respectively), Fraction B and the starting material (fraction A) differed sufficiently in polarity to allow their separation by TLC. The intense band at 990 cm^{-1} in

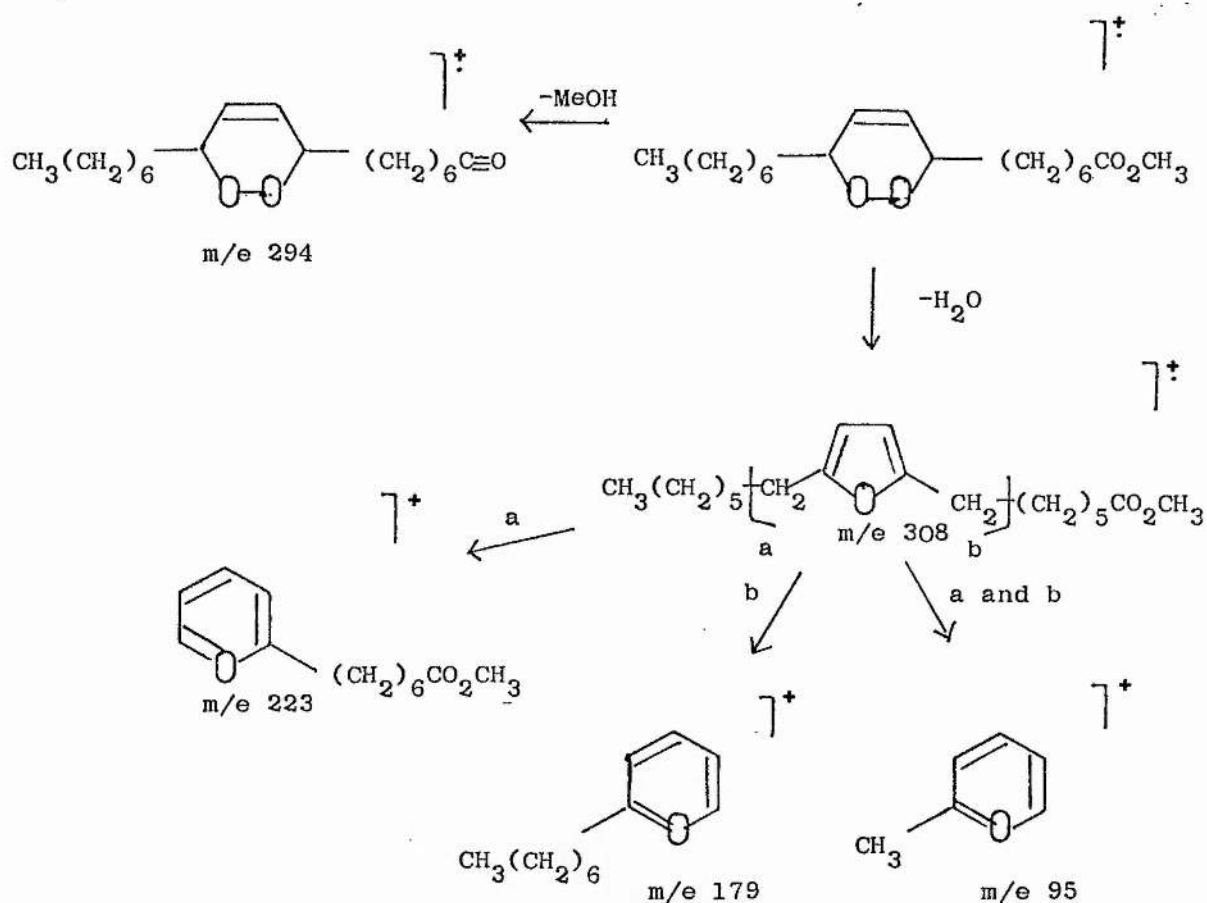
the IR spectrum of the starting material was replaced by one at 790 cm^{-1} in that of B. A two proton signal at 4.32 in the PMR spectrum of B is assigned to hydrogens attached to



carbon atoms adjacent to the oxygens (C-8 and C-11) and the singlet at 5.80 to the two olefinic hydrogens. The mass spectrum of B did not show its molecular ion (326) but exhibited peaks at m/e 308 and 294, which we identify as ions due to loss of water (M-18) and methanol (M-32) respectively from the parent ion. Enzell *et.al.*³²⁰ observed a similar dehydration during mass spectroscopy of the diterpenoid endoperoxide (121), giving the furan (122). The ion so formed then undergoes normal fragmentations



of furanoid compounds. Thus, the endoperoxide B displayed the following peaks.



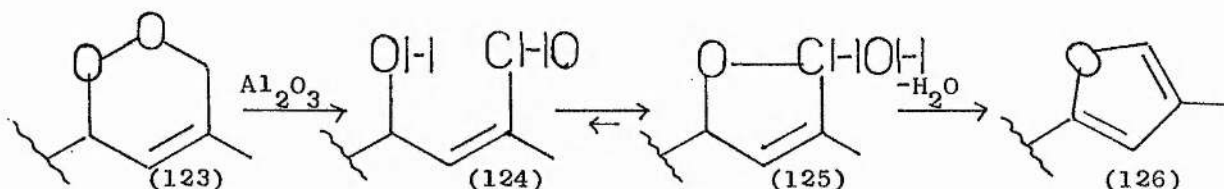
Minor peaks representing the 9,12-endoperoxide were also present, and we believe that this isomer was formed from methyl octadeca-trans-9,trans-11-dienoate which might have been a minor contaminant of the starting material.

Fraction C probably consisted of hydroperoxides formed by 'ene' reaction but these were not investigated.

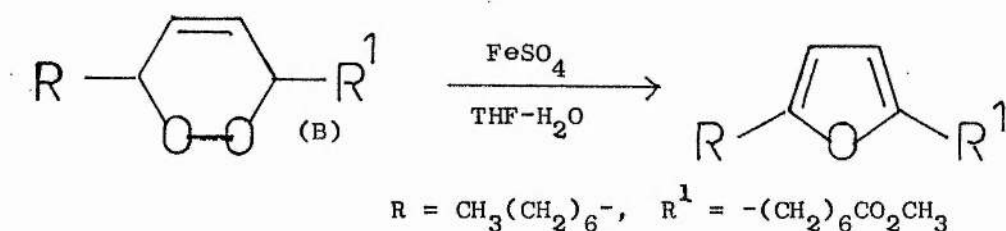
The oxygenation of acyclic conjugated dienes with singlet oxygen to afford 1,4-endoperoxides reported by other workers are limited to terminal dienes. Our successful preparation of a mid-chain endoperoxide (B) in excellent yield is, therefore, worthy of note.

(b) Reduction of endoperoxide to furan

The classical method of dehydrating an endoperoxide to a furan is that of Kornblum and DeLaMare³²¹ whereby contact with basic alumina rearranges endoperoxide (123) to hemiacetal (124). The latter is readily dehydrated



to furan (126) upon injection into a gas chromatograph³²¹ or when chromatographed on silica³²². Kondo and Matsumoto in 1972, described a procedure involving treatment of the endoperoxide with lithium *t*-butoxide followed by dehydration with sulphuric acid²⁹⁸. More recently Turner and Herz³⁰⁴ reported that this reduction is effected in excellent yield by treatment of the endoperoxide with ferrous sulphate in aqueous THF. We used this simple method to reduce the endoperoxide (B) and confirm the findings of Turner and Herz. It was necessary, however, to use ferrous sulphate and endoperoxide in a molar ratio of 2:1 which is higher than that used by Turner and Herz (1.5:1). We also found that the reduction is improved in more aqueous solution, and obtained 72% conversion in 5 h. The rest



was unreacted endoperoxide and no by-products were observed.

Turner and Herz suggest that the endoperoxide is converted to a hemiketal via one or more radical anion intermediates, which then undergoes dehydration to give the furan.

In view of the excellent yields obtained both during formation of endoperoxide and its subsequent dehydration, this method appears to be useful in the syntheses of furan. Its value is limited only by the availability of the appropriate dienes. The biogenetic implications of this reaction are discussed in Section I (xi).

Section III: CHEMICAL REACTIONS AND SPECTROSCOPY

INTRODUCTION

Because of the novelty of long-chain furanoid acids little is known about their chemical reactions, and in this section we report some reactions of 10,13-furan acid and its methyl ester. Also included is a discussion on the application of spectroscopic methods for the recognition and identification of long-chain furanoid acids.

RESULTS AND DISCUSSION

(A) Chemical Reactions

1. Acid-Catalysed Ring-Opening

Lability of furans towards acids is well known. We studied the reaction of 10,13-furan with acids under various conditions, namely, (i) hot and (ii) cold H_2SO_4 , (iii) BF_3 -MeOH. The 10,13-diketone was readily produced under all these conditions resulting in an equilibrium with the furan (Table 32) [approx. 2:3 (diketone to furan)].

Table 32: Acid-catalysed Equilibrium Between 10,13-furan and Diketone

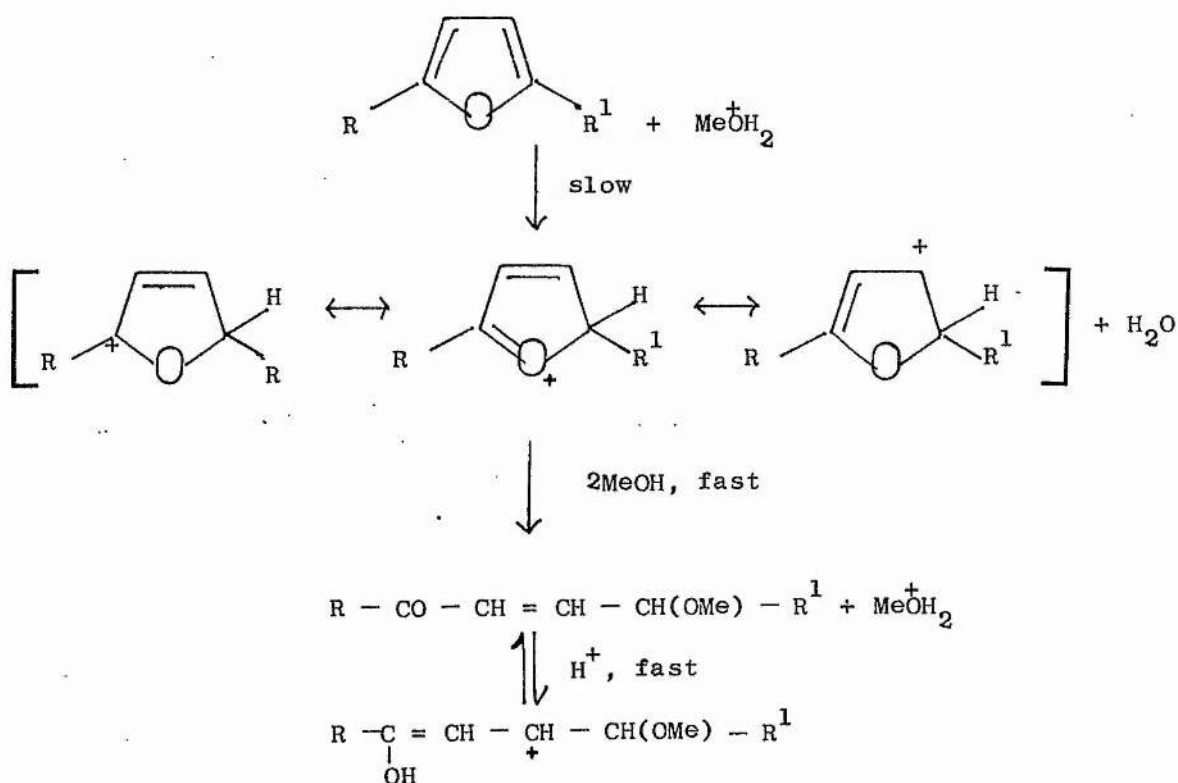
	Cold H_2SO_4 -MeOH with furan	Hot H_2SO_4 -MeOH with furan	Hot BF_3 -MeOH with furan	Cold H_2SO_4 -MeOH with diketone
furan	59*	57	68	51
diketone	41	43	32	45
other	-	-	-	4

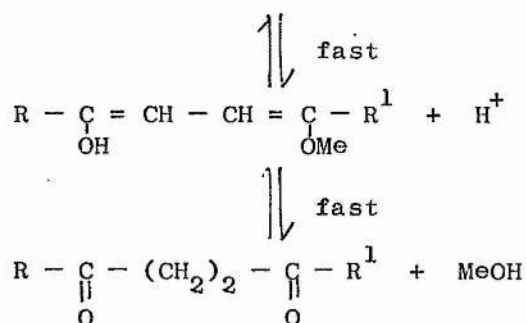
*The relative amounts of the furan and diketone were calculated by GLC and are given as weight %.

The reaction with $\text{BF}_3\text{-MeOH}$, which was carried out for only 20 min, appears to give slightly more furan than with other catalysts but further experiments are necessary to check this. Results reported with $\text{H}_2\text{SO}_4\text{-MeOH}$ are for 1 h reaction, and longer reaction times did not alter the equilibrium.

The net result of the ring-opening is hydrolysis. Stamhuis *et.al.*³²³ carried out a kinetic study of the hydrolysis of furan and 2,5-dimethylfuran and showed that the reaction involves protonation at the α -carbon of the furan ring and not on the oxygen. This protonation is the rate-determining step, and this then allows nucleophilic attack by the solvent. Our reactions were carried out in dry methanol and we believe that the reaction takes place by a mechanism analogous to that proposed by Stamhuis *et.al.*:

Scheme 5: Mechanism for interconversion of furan and diketone by acid





When 9,12-dioxostearic acid was treated with hot methanolic sulphuric acid a similar furan:diketone equilibrium was established. The mechanism of dehydration may be the reverse of that by which diketones are formed (Scheme 5).

In view of the fact that during the isolation of natural F acids by procedures involving urea crystallization the urea mother liquor is acidified before extraction, we carried out a similar operation with the synthetic 10,13-furan. No diketones were formed during this quick operation but a product (11%) slightly more polar than the furan ester was detected (ECL 22.6, SP 2300). We did not characterize this compound.

The ease of ring-opening of furans may be used as a method of obtaining γ -diketones, whose preparation by presently available procedures is no easy matter²²⁶⁻²³⁹. γ -Diketones are important starting materials for many classes of compounds such as cyclopentenones and pyrroles. Although furans themselves are conventionally made from γ -diketones, preparation of the diketone by the reverse reaction may be convenient when an efficient alternative route to the corresponding furan is available. For example, 10,13-dioxostearate can be prepared from 10,13-furan, which

is readily obtained from methyl vernolate or 12,13-dihydroxy-oleate. When long-chain 2,5-disubstituted furans contain an additional methyl substituent on the ring the position of that group cannot be fixed by mass spectrometry of the furan. Acid-catalysed ring-opening followed by mass spectrometry of the resulting diketone may provide a simple method of determining the position of the methyl substituent.

2. ^dHydrogenation

The catalytic hydrogenation of furan and its derivatives is strongly dependant on reaction conditions and catalysts employed - hydrogenolysis of the ring being an important side-reaction³²⁴. Hydrogenation of 10,13-furan at room temperature and pressure using 10% palladized charcoal gave the corresponding THF ester in good yield and we did not detect any products due to ring-opening. GLC of the resulting THF esters showed two peaks (3:1) and we believe that these are due to the cis and trans isomers.

The method used by Glass et.al.^{11,12} for the isolation of natural F acids involves hydrogenation and we were interested to know whether any non-methylated F acids could survive the reaction conditions. We attempted the selective hydrogenation of methyl oleate when present with 10,13-furan. Reaction using 10% Pd/C as catalyst resulted in simultaneous hydrogenation of the furan ring. Adams catalyst proved to be unselective too. Recently Lie Ken Jie et.al.²¹² used Lindlar catalyst for the selective hydrogenation of unsaturated side chains attached to furan.

Adams catalyst is useful for the hydrogenation of methyl oleate without affecting the furan ring in the natural (fish) F acids. We added methyl oleate to an extract of F acids (F_1 - F_6) and hydrogenation of this mixture (in methanol solution) for 20 min. using Adams catalyst caused little change in the furans while methyl oleate was completely hydrogenated. However, the reaction time is critical in this operation and complex products result by prolonged reaction.

3. Attempted Diels-Alder Reaction

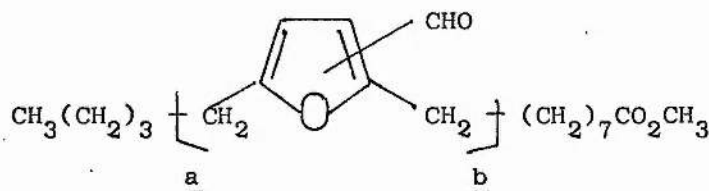
Diene syntheses using furan, to form a bicyclic molecule in a 1,4-cycloaddition reaction with a dienophile are well known. A similar reaction with long-chain furans is of interest because it may be developed as the basis of a convenient isolation procedure for natural F acids.

Shorter-chain conjugated dienes adduct with maleic anhydride in the cold but the 18:2(81101) ester required heating with benzene for 4 h. Furan itself reacted with maleic anhydride after 2 h with refluxing benzene and these results indicated that long-chain furans react much more slowly. After a nine hour reaction of 10,13-furan acid with maleic anhydride in refluxing benzene, no adduct was detected and the furan was recovered unchanged. Use of a higher reaction temperature by conducting the reaction in toluene gave the same result.

4. Formylation

The F acids occurring in fish lipids contain either one or two methyl groups on the β -positions of the ring. Having developed convenient routes to 10,13-furan we were interested in converting this compound to its mono- and di-methyl derivatives, F_2 and F_3 respectively. Direct alkylation of the β -positions of furans is not practicable, so we tried to prepare the methyl derivatives of 10,13-furans via formyl intermediates.

Treatment of the 10,13-furan (ECL 20.7) with Vilsmeier reagent³²⁵ resulted in the slow formation of two products of ECL 25.8 and 28.0. Heating of this reaction mixture on a steam bath diminished the products already obtained. Therefore, the formylation was carried out at room temperature for 64 h, when all the starting material had reacted. The GLC peak of ECL 25.8 was ascribed to a mixture of the two monoformyl derivatives, on the basis of the following evidence. Its mass spectrum showed the molecular ion at 336 and contained intense peaks at m/e 307 and 308 which were assigned to ions arising from loss of $-CHO$ (c), followed by hydrogen abstraction (c+1) respectively. The base peak (m/e 151) is due to loss of $-CHO$ from the



fragment ion (b). Ions due to (b) and (b+1) were also present (m/e 179 and 180). The monoformyl structure was further evidenced by the presence of a peak at m/e 123 due to double β -cleavage (a+b) and one at m/e 95 due to loss of water from it. The PMR did not contain a signal at 5.70 (furanoid H) but showed two doublets at 5.9 and 6.0 instead. We have assigned these signals to the 3- and 4-hydrogens in the monoformyl furan derivatives. We suspect that the second product (ECL 28.0) is the diformyl derivative but the available information (see Experimental Section) is not sufficient to confirm this assignment.

Attempted reduction of the monoformyl derivative

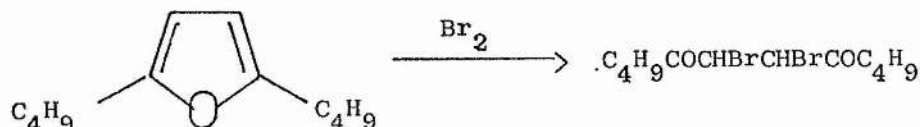
Reduction of the 3-formyl derivative of 10,13-furan was expected to furnish F_2 . We attempted this conversion via the tosylhydrazone of the aldehyde³²⁶ but failed to obtain the desired product. The reaction product showed IR bands at 3180 (N-H) and 810 (1,4-disubstituted benzene) showing that it was comprised of the hydrazone. Tosylhydrazones of aliphatic aldehydes and ketones are reduced efficiently to the corresponding methyl derivatives by $NaBH_4$ and the failure in the present case may be due to the aromatic character of the furan aldehyde³⁸⁷.

5. Attempted Bromination

Bromine was readily decolourized by 10,13-furan until an equimolar quantity had been added. Identification of the reaction products, however, proved to be difficult. The reaction product which showed a major (GLC) peak of ECL

22.8. deposited a precipitate on standing and neither the precipitate nor the liquor gave a peak at ECL 22.8.

Brown and Wright³²⁸ found that 2,5-dibutylfuran is ring-opened by reaction with bromine;



and it is possible that the precipitate formed with 10,13-furan is due to an analogous reaction.

6. Oxidation

Long-chain furans (both natural and synthetic) do not keep well even when stored at sub-zero temperatures in petrol solution, and we believe that this lability is due at least in part, to autoxidation. Set out in Table 33 are the relative amounts of P and F esters in a fresh and 10 month-old extract. All the furan esters (F₁-F₇) are labile

Table 33: Change in Composition of F Esters During Storage

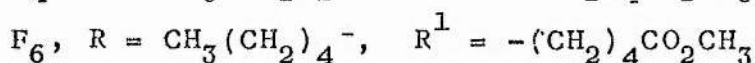
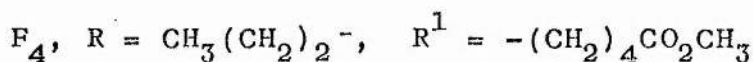
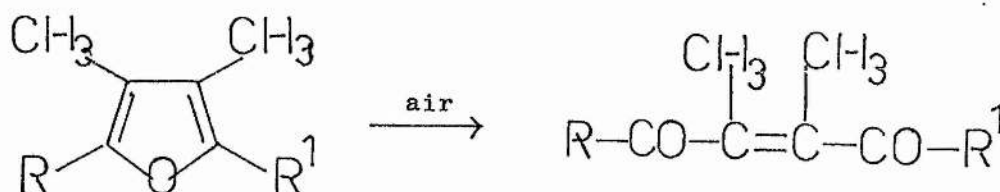
<u>Composition</u>	<u>Fresh Extract</u>	<u>10 Month-old Extract</u>
P ₁₆	9.6	19.7
P ₁₉	4.6	9.8
P ₂₀	14.9	34.4
F ₁	1.5	1.1
F ₂	4.8	4.7
F ₃	2.8	1.8
F ₄	12.6	8.5
F ₅	4.7	4.9
F ₆	42.6	14.2
F ₇	1.9	0.9

relative to the P esters, F₄ and F₆ being the most so. The sensitivity of the F esters to oxygen was demonstrated by exposing a mixture of natural P and F esters to a stream of oxygen continually drawn through it. The composition of this mixture at different periods are shown in Table 34.

Table 34 : Change in Composition of F Esters by Exposure to Oxygen.

<u>Composition</u>	<u>Start</u>	<u>2 Days</u>	<u>14 Days</u>	<u>21 Days</u>
P ₁₆	8.6	9.7	14.3	16.6
P ₁₉	14.5	15.8	22.7	25.0
P ₂₀	32.8	34.3	46.8	55.7
F ₁	0.8	0.6	0.6	-
F ₂	4.4	3.5	3.8	1.4
F ₃	1.8	1.5	0.4	-
F ₄	6.2	5.4	1.7	-
F ₅	3.0	2.6	2.7	0.8
F ₆	27.9	26.6	7.0	0.5

The composition of the mixture after 14 days clearly shows the quicker reaction of F₄ and F₆ than that of the other members. The enhanced reactivity of F₄ and F₆ may be attributed to their dimethyl substituted furan structure. It has been shown³²⁸ that 2,5-di-tert-butylfuran is transformed into 1,2-diacylethylene via atmospheric oxidation and we believe that the lability of our F esters is also due to similar ring-opening. Thus:



The enhanced reaction with F_4 and F_6 may be due to the greater stability of the tetrasubstituted double bond in the resulting diketone.

(B) Spectroscopic Identification of Long-chain Furans

(i) IR Spectroscopy

The IR spectra of 2,5-disubstituted long-chain furans exhibit several sharp peaks which readily distinguish them from the more common fatty acids which do not contain this heterocyclic system. They are:

- | | | | |
|-----|------------------------|-------|--------------------------------------|
| (a) | 3100 cm^{-1} | (f,w) | C-H stretching in the furan |
| (b) | 1610 cm^{-1} | (f,w) | asymmetrical C=C stretch |
| (c) | 1560 cm^{-1} | (f,s) | symmetrical C=C stretch |
| (d) | 1010 cm^{-1} | (f,s) | ring breathing |
| (e) | 775 cm^{-1} | (f,s) | out-of-plane deformation of the ring |

The following IR absorptions have been reported¹² for the 3-methyl and 3,4-dimethyl derivatives of 2,5-disubstituted long-chain furans.

	3-Methyl derivatives (cm^{-1})	3,4-Dimethyl derivatives (cm^{-1})
(b)	1630 cm^{-1}	1645
(c)	1570 cm^{-1}	1590
(d)	1025 cm^{-1} (w)	-
(e)	785 cm^{-1} (w)	-

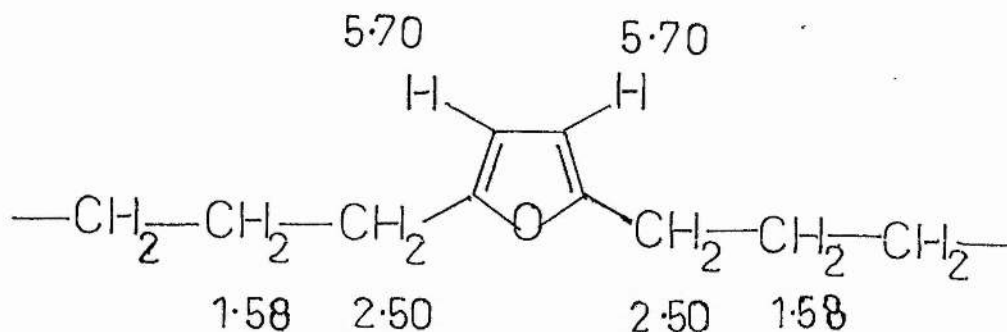
The trisubstituted ring shows weak peaks at 1025 and 785 cm^{-1} , which are absent from the spectra of tetrasubstituted furans. Symmetrical and asymmetrical C=C stretching vibrations appear at 1560 and 1610 cm^{-1} for 2,5-disubstituted furans. These frequencies are raised to 1570 and 1630 for their 3-methyl derivatives and to 1590 and 1645 cm^{-1} for the 3,4-dimethyl derivatives, suggesting increased double bond strength with additional substitution.

(ii) UV Spectroscopy

The UV spectra of 2,5-disubstituted furans show a maximum at 222 nm, $\epsilon = 9,000$ (hexane), and the tetrasubstituted ring in F_6^{12} absorbs at 227 nm, $\epsilon = 7400$. Some conjugated dienes also absorb in this region but generally they have a higher molar extinction coefficient ($\sim 23,000$).

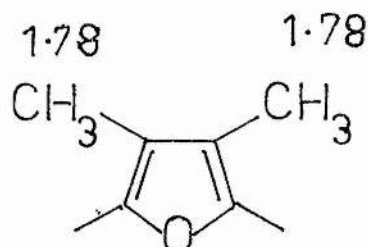
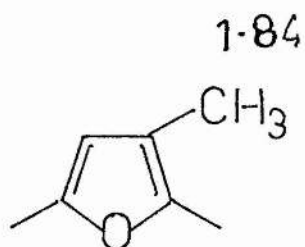
(iii) NMR Spectroscopy

Signals in the ^1H nmr spectra of 2,5-disubstituted furans at 5.70(s), 2.50(t) and 1.58(m) are of diagnostic value.



The singlet at 5.70 is due to the hydrogens in ring positions 3 and 4. The triplet at 2.50 arises from the α -methylenes on either side of the furan ring, and is well separated from the triplet due to the methylene next to the ester group (2.20). The β -methylene protons appear at 1.58 close to the multiplet due to the other chain methylene groups.

It has been reported¹² that the protons of the methyl groups on the ring in F_5 (one methyl group) and F_6 (two methyl groups) cause a singlet at 1.80. Though we have not examined the PMR spectra of individual F_5 or F_6 , we have observed two singlets at 1.78 and 1.84 in the PMR spectra of concentrates of F_1 - F_7 . These may be due to

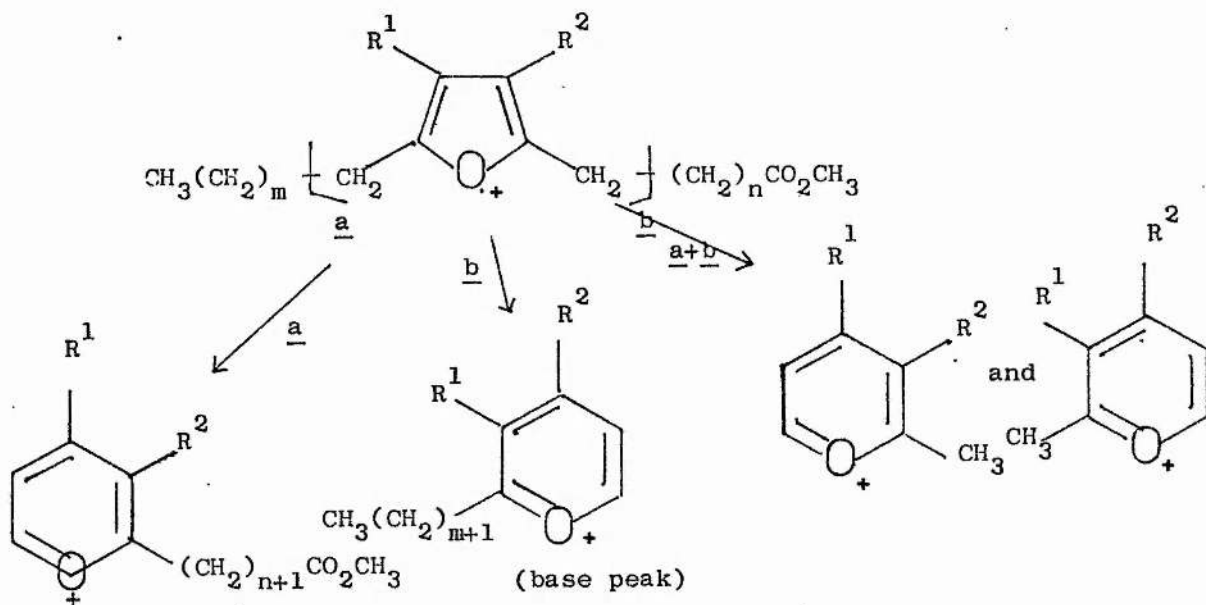


slightly different nuclear resonances of the methyl protons in the monomethyl and dimethyl derivatives.

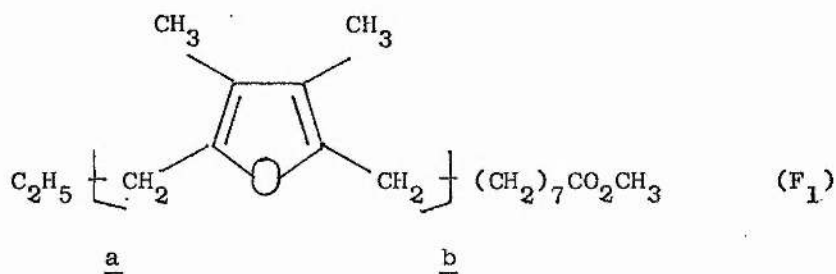
^{13}C NMR spectroscopy of long-chain furans are less well established. For 10,13- furan (C_{18}), the pertinent features are signals at 154.6 and 154.5 (ring C-2 and C-5) and at 104.9 (C-3 and C-4).

(iv) Mass Spectrometry

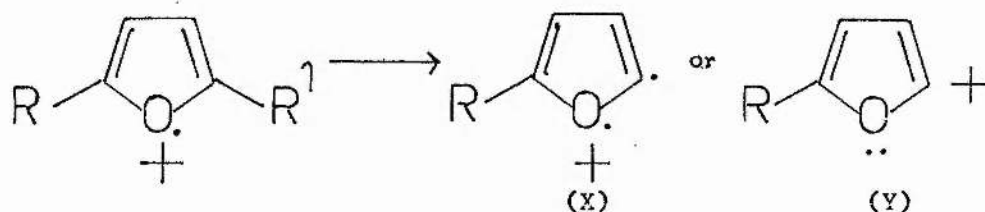
Mass spectrometry is probably the most important method of identifying long-chain furans. Unlike the conventional fatty acid methyl esters, long-chain furans produce simple mass spectra with a few intense peaks and an abundant molecular ion is usually observed. Consideration of the two prominent β -cleavage ions a and b together the fragment arising from double β -cleavage (a+b) allows the position of the furan ring in the alkyl chain to be unambiguously fixed. The a+b ion also indicates the number of additional carbon atoms attached to the ring. For example, F_1 has the molecular ion at 308 ($\text{M}, 20$) and the base peak at



151(b) shows that it is a 10,13-isomer. The peak at 279 (a,23) indicates n-4 structure. This is confirmed by the presence of the double β -cleavage ion at 123 (a+b,14) which also shows that the ring is dimethylated.



Fragment b is always the base peak, cleavage α to the furan ring does not occur or is negligible, presumably because it leads to unstable vinyl or diradical ions (Y and X), but γ -cleavage ions are usually observed.



The tetrasubstituted derivatives (having two methyl substituents) show minor M-CH₃ peaks. They generally exhibit more intense ion fragments than do the monomethyl derivatives (Table 35), and ion a in F₂ and F₅ is relatively small. The nonmethylated 2,5-disubstituted furanoid esters also show ions at *m/e* 81(c) and 69(d).

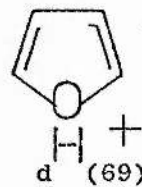
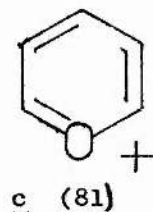


Table 35: Mass Spectrometry of Natural Long-Chain Furanoid Methyl Esters.

Monomethyl derivatives

(<u>n</u> -6)	<u>M</u>	<u>M-15</u>	<u>M-31</u>	<u>a</u>	<u>b+14</u>	<u>b</u>	<u>a+b</u>
F ₂	322(18)	-	291(4)	265(7)	179(2)	165(100)	109(14)
F ₅	350(22)	-	319(4)	293(8)	179(2)	165(100)	109(18)

Dimethyl derivatives

(<u>n</u> -6)							
F ₃	336(36)	321(2)	305(9)	279(41)	193(4)	179(100)	123(23)
F ₆	364(38)	-	333(6)	307(31)	193(4)	179(100)	123(22)

Dimethyl derivatives

(<u>n</u> -4)							
F ₁	308(20)	293(1)	277(5)	279(23)	165(4)	151(100)	123(14)
F ₄	336(19)	321(1)	305(5)	307(19)	165(5)	151(100)	123(14)
F ₇	364(52)	-	333(10)	335(26)	165(9)	151(100)	123(19)

EXPERIMENTAL: GENERAL

I. SOLVENTS

Solvents were of reagent grade, and redistilled before use. Dimethylformamide and dimethyl sulphoxide were dried by refluxing with calcium hydride for 2 hr before distillation and the dry solvents were stored over molecular sieve type 3A ($\frac{1}{8}$ " pellets). Pyridine was dried similarly using potassium hydroxide instead of calcium hydride and stored over potassium hydroxide pellets. Benzene was dried by azeotropic distillation and stored over sodium wire.

II. CHROMATOGRAPHIC ANALYSIS

(i) Thin Layer Chromatography

Analytical TLC was carried out on glass plates (20 x 5 cm) coated with silica gel G (0.25 mm wet thickness). Where a high resolution was not required microscope slides, coated by dipping in a silica gel G-chloroform slurry and dried in air, were used. For separations on a preparative scale glass plates (20 x 20 cm) were coated with silica gel G (1.0 mm, wet thickness). After their preparation the TLC plates were dried at room temperature for 1 hr, activated at 110-120° for 2 hr and stored in a drying cabinet containing silica.

Mixtures of petroleum (bp 40-60°) and diethyl ether were normally used as developing solvents for TLC. Abbreviations such as PE20 indicate mixtures of petroleum

and diethyl ether in a ratio of 80:20 (v/v). A little acetic acid added to the developing solvent was useful when acids were separated. A typical solvent system employed for such purpose was PEA(80:20:1), which stands for a mixture of petrol diethyl ether and acetic acid in the ratios indicated.

The components on analytical TLC plates were generally detected by spraying with an ethanolic solution of phosphomolybdic acid (10%, w/v) and then heating at 110-120°. Spots, due to long-chain unsaturated or oxygenated compounds, usually appeared within 5 minutes but saturated compounds required further heating.

Peroxides appeared as red-brown spots after spraying with a reagent prepared by dissolving ferrous sulphate (4 g) and ammonium thiocyanate (4 g) in hydrochloric acid (1 M, 70 ml). The spray reagent was decolourized prior to use by washing with pentan-1-ol. The peroxide spots fade quickly and were marked before the phosphomolybdic spray was applied to show other compounds.

Preparative TLC plates were sprayed with an ethanolic solution of 2',7'-dichlorofluorescein (0.2%, w/v) and viewed under ultraviolet light. The resolved components which appeared as yellow bands in a purple background were marked, scraped off the plate, and extracted with diethyl ether. The solvent was removed under reduced pressure and the last traces were blown off with nitrogen. The separated bands are labelled as A, B, C etc. in the order of decreasing R_f values.

Column chromatography was used for purification of more than 1 g of material. The column was packed with Sorbsil (a silica gel preparation, mesh 60) or 30% silver nitrate in Sorbsil using 20-25 g of adsorbant per gram of material to be separated. The components were usually eluted with PE mixtures containing progressively more diethyl ether, and the separation was monitored by TLC and/or GLC.

(ii) Gas Liquid Chromatography

GLC was carried out on a Pye series 104 chromatograph equipped with a flame ionization detector. The glass columns (1.5 m long, 6 mm external diameter) containing five different stationary phases: (i) 20% DEGS, (ii) 20% EGSS-Y, (iii) 10% SP-222-PS, (iv) 10% SP 2340 and (v) 10% SP 2300, coated on Chromosorb W AW (100-120 mesh) supports were used. (The packing materials were obtained from SUPELCO, INC.). In the case of the first three columns the oven temperatures employed were 185°, 190° and 190°C respectively. SP 2340 and SP 2300 columns were used at more than one temperature (up to a maximum of 250°C) but unless a statement is made to the contrary, it should be assumed that they were employed at 195° and 240°C respectively. Nitrogen was used as carrier gas at a flow rate of 60-70 ml min⁻¹ and was dried by passage through molecular sieve type 4A, especially when using the SP-222-PS column. Quantitation of the peaks was based on peak height x retention distance and compositions of mixtures are reported as weight percentages. Saturated

straight chain methyl esters were used as external or internal standards and, the ECL values and percentage areas were calculated by computer. Apparent inconsistencies in ECL values reported in the text are due either to different operating temperatures or to the deterioration of the polar liquid phase with prolonged use. Authentic samples of polyunsaturated and oxygenated fatty acid methyl esters were chromatographed periodically to check the variation of ECL values with the ageing of the column.

III. SPECTROSCOPY

IR spectra were recorded on a Perkin Elmer 257 grating spectrophotometer. Unless stated to the contrary, samples were run as films between sodium chloride discs. The absorption frequencies are reported in cm^{-1} and are accurate to $\pm 5 \text{ cm}^{-1}$.

UV spectra were recorded on a UNICAM SP 800 B spectrophotometer. Samples were run in hexane solution using silica quartz cells, 1 cm wide.

Mass spectra were recorded with direct-probe insertion of samples into the source of an AEI MS 902 mass spectrometer. The source pressure was 2×10^{-7} torr, source temperature about 200°C , and the ionization voltage 70 eV. A silicone membrane separator was used for GC-MS studies.

PMR spectra were recorded at 100 MHz on a Varian HA100 instrument using 10-15% solutions in carbon tetrachloride with 3% tetramethylsilane as the internal

standard. Chemical shift values are given in ppm downfield from tetramethylsilane ($\delta=0$).

Natural abundance ^{13}C NMR spectra were obtained with a Varian CFT-20 spectrometer in the Fourier transform mode of 20 MHz, with proton noise decoupling (1 KHz band width). The samples were run in deuteriochloroform solution (~2 ml) with tetramethylsilane (3%, v/v) added as the internal standard. Whenever possible 1 M solutions were used but when only small quantities of material were available (50-200 mg), the 0-200 ppm spectrum was obtained by overnight scanning. The sample was usually spun at 20 rps, at room temperature, and the spectrometer's computer printed out peak heights and chemical shifts (ppm downfield from tetramethylsilane).

IV. MELTING POINTS

Melting points were determined on Gallenkamp apparatus and are uncorrected.

General Chemical Procedures

1. Saponification

The lipid (1 g) was refluxed with a 1 M solution of potassium hydroxide in 95% ethanol (6 ml) for 1 hr. After cooling the reaction mixture, water (12 ml) was added and the solution extracted with diethyl ether (3 x 10 ml) to remove the nonsaponifiable material. The aqueous layer was then acidified with hydrochloric acid (6 N) and extracted with ether (3 x 10 ml). The free fatty acids were recovered by washing the extract with water, drying it over anhydrous sodium sulphate and removing the solvent

under reduced pressure.

2. Esterification

Free fatty acids were usually esterified with hot methanolic sulphuric acid. The acid (1 g) was refluxed with methanolic sulphuric acid (2% w/v, 30 ml) for 2 hr and allowed to cool. Brine (5%, 40 ml) was added to the reaction mixture which was then extracted with ether (2 x 30 ml). The combined ether extracts were washed with brine solution, dried over anhydrous sodium sulphate, and the solvent removed under reduced pressure to obtain the methyl esters.

3. Transesterification

In the majority of cases reported in this thesis, and particularly when examining for furanoid acids, esters were prepared by transesterification of the lipid with sodium methoxide in methanol, according to the following procedure.

The lipid (1 g) was dissolved in benzene (3 ml) and refluxed with sodium methoxide in methanol* (0.5 M, 6 ml) for 45 min. The esters were recovered in the usual way as described above for the esterification with hot methanolic sulphuric acid. This procedure was scaled up where necessary, for example, dogfish liver oil (200 g) was esterified with sodium methoxide (0.5 M, 500 ml) for

* This reagent stored at 0° was stable up to 3 months.

the purpose of isolation of furanoid fatty esters.

4. Hydrogenation

The unsaturated ester (10 mg) was dissolved in methanol (10 ml) in a RB flask (25 ml) and palladium on charcoal (20%, 10 mg) added. The flask was connected via a two-way tap to a rubber balloon filled with hydrogen and to a water-pump. The flask was alternatively evacuated and flushed with hydrogen several times to remove any air, and finally opened to the hydrogen atmosphere. The reaction mixture was then stirred vigorously with a magnet for 1 h, at the end of which time the solution was filtered to remove the catalyst and the solvent removed to recover the hydrogenated ester.

5. Preparation of trimethylsilyl ethers

The hydroxy compound (10 mg) was shaken with pyridine (1 ml), hexamethyldisilazane (0.3 ml) and trimethylchlorosilane (0.1 ml) for 30 sec. After standing for 5 min, water (5 ml) was added and the solution extracted with ether (2 x 5 ml). The combined ether extracts were dried over anhydrous sodium sulphate and the solvent removed in a stream of nitrogen until the odour of pyridine was gone.

6. Sodium borohydride reduction

The keto-ester (10 mg) in dimethylformamide (1 ml) was shaken with sodium borohydride (10 mg) at room temperature. After 1 h, water (2 ml) was added and the organic product extracted with ether (3 x 2 ml). The combined

ether extracts were dried over anhydrous sodium sulphate and the hydroxyester was recovered by removal of the solvent under reduced pressure.

EXPERIMENTAL: Section I -- ANALYTICAL

I. SOURCE OF EXPERIMENTAL SAMPLES

(a) Fish

The freshwater fish were obtained from Loch Lomond in South West Scotland. Apart from the female roach (October, 1976) and the pike (August, 1976), the fish were captured in October, 1975. The salmon was caught in a river off the west coast of Scotland, in August, 1975. The octopus was obtained from the Gatty Marine Laboratory (St. Andrews) in April, 1976 and the ice fish was captured in the Antarctic (June, 1976). The haddock, herring, and mackerel were caught in the North Sea off Aberdeen. Capelin, dogfish, and one of the cod liver oil samples were commercial refined oils obtained from Marfleet Refining Company, Hull. The remaining cod liver analyses [referred to as cod (fed) and cod (starved)] were carried out on samples obtained from fish maintained in captivity at the Torry Research Station, Aberdeen.

(b) Others

The fish meals were obtained from commercial sources. Eggs were bought from a supermarket. The Exocarpus cupressiformis seeds were supplied by the CSIRO, Australia.

II. EXTRACTION OF LIPIDS FROM ANIMAL TISSUES

Tissue was stored at -20° under methanol containing 2,6-di-tert-butyl-p-cresol (BHT) (50 mg/l of solvent) and lipid was extracted when required, according to the following procedure.

The tissue (25 g, wet weight) was homogenised in a blender (MSE, London) with methanol (50 ml) and chloroform (25 ml) for 2 min. (Where the sample had been stored in methanol, the same solvent was used for extraction). More chloroform (25 ml) followed by water (25 ml) was added and the blending continued for 30 sec after each addition. The homogenate was filtered through a sintered glass funnel under reduced pressure. Normally, the filtration is quite rapid and when the residue became dry it was pressed to ensure maximum recovery of filtrate. The filtrate was then transferred to a separating funnel and after allowing sufficient time (~ 15 min) for clarification the chloroform layer was separated, and dried over anhydrous sodium sulphate. Removal of the solvent under reduced pressure in a rotary film evaporator gave the purified lipid

This procedure is based on the assumption that the tissue contains approximately 80% of water; and is suitable for the extraction of lipid from wet livers. It is important that the final ratio of chloroform, methanol, and water be as close to 2:2:1.8 (by volume) as possible. When extracting lipids from fish meals more water was added to obtain this solvent ratio. The lipid contents quoted in Table 11 are the weights of lipids as a percentage of the

weight of the wet tissue.

III. EXTRACTION OF LIPIDS FROM VEGETABLE SEEDS

The dry, crushed seeds contained in a Soxhlet were extracted with petroleum-ether (bp 40-60⁰) for 2 h. The seeds were then re-ground and extraction continued for another 2 h. The total extract was filtered and the solvent removed on a rotary film evaporator to obtain the lipid.

IV. SEPARATION OF LIPID CLASSES

(i) Column Chromatography

Lipids of different kinds were separated by silica acid column chromatography, (25 g Sorbsil/g of lipid). Petroleum ether (bp 40-60⁰) containing increasing quantities of diethyl ether was used as the eluting solvent. Hydrocarbons and pigments (when present) eluted with petrol, the cholesterol esters with PE3 and the triacylglycerols with PE10. The column was finally eluted with methanol to furnish the polar lipids. The separation was monitored by TLC.

(ii) Preparative Thin Layer Chromatography

Prep. TLC was used in cases where the quantity of lipid to be separated was small (<1 g) and to purify fractions obtained by column chromatography. Often cholesterol esters were purified in this way. Up to 50 mg of lipid was chromatographed on one plate using PEA (80:20:1) as developing solvent and the lipids were detected in the usual way.

A ferric chloride spray prepared and used as follows was valuable to detect cholesterol esters and free cholesterol on TLC plates .

Ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 50 mg) was dissolved in water (90 ml) with acetic (5 ml) and sulphuric (5 ml) acids. The developed TLC plate was sprayed with the reagent and heated at 100° for 2-3 min. The presence of cholesterol esters and free cholesterol was indicated by the appearance of a red-violet colour. The colour of cholesterol appeared slightly before that of its ester. Lipid of egg yolk was a useful reference standard for cholesterol esters and free cholesterol.

V. UREA FRACTIONATION

Esters (1 g) were crystallized overnight at 0° from a solution of methanol (30 ml) containing urea (5 g). The precipitate was filtered under reduced pressure and washed with cold methanol (3 ml) saturated with urea. Most of the methanol was removed from the filtrate under reduced pressure in a rotary film evaporator and, after dilution with water (25 ml) and acidification with dilute hydrochloric acid the esters were extracted with diethyl ether (2 x 25 ml). The combined ether extracts were washed with water (5 ml), dried over anhydrous sodium sulphate, and the solvent removed under reduced pressure to obtain esters which did not adduct with urea (Mother Liquor). The precipitate was dissolved in water (50 ml) and extracted with ether (2 x 30 ml). Work up, as described for the recovery

of the mother liquor, furnished the esters which formed adducts with urea (Urea Adduct).

VI. ARGENTATION CHROMATOGRAPHY

(i) Ag⁺ Thin Layer Chromatography

Ag⁺ TLC was carried out on plates (20 x 20 cm) coated with silica gel G (1 mm thick) containing silver nitrate (10%). The plates were activated at 110° for 2 h after preparation, and if not used immediately, they were stored in a desiccator protected from light, and reactivated at 110° for ½ h before use. PE mixtures (usually PE30) were used as developing solvent. After development the plates were dried in a gentle stream of nitrogen and sprayed with an ethanolic solution of 2',7'-dichlorofluorescein (0.2%). The separated components appeared as yellow bands on a purple background when viewed under ultraviolet light. These bands were scraped off and the esters were extracted with ether.

This procedure was modified for the isolation of furanoid and phytol-based esters which travel together or very close to each other. A sample of furanoid esters extracted from a fish oil was run along one edge of the plate at the same time as the sample under study was being chromatographed. With only the edge strip exposed, the plate was sprayed with phosphomolybdic acid (10% ethanolic solution). A dark brown spot developed at room temperature within 1-2 min denoting the location of the 'furan band'.

(ii) Ag⁺ Column Chromatography

Silica acid impregnated with silver nitrate was prepared as follows.

Sorbsil (60 mesh, 250 g) and an aqueous solution of silver nitrate (50%, 150 ml) were slurried together and mixed thoroughly. It was then activated overnight at 110°. The adsorbent was packed into the chromatographic column in the conventional manner except that the column was wrapped in aluminium foil to exclude light. Phytol-based esters and furanoid esters were readily isolated from concentrates not containing saturated esters by elution with PE5.

VII. DETAILED EXAMINATION OF DOGFISH LIVER OIL

Dogfish liver oil methyl esters (100 g) and urea (500 g) were crystallized from a mixture of methanol (0.67 l) and ethanol (1.33 l) and the mother liquor (11 g) chromatographed on silica gel impregnated with silver nitrate (30%, 250 g). The column was eluted first with PE5 and then with PE10, collecting 100 ml fractions. Elution with PE5 (fractions 1-7) furnished F. esters, branched-chain esters, and two unidentified components (designated U₁ and U₂), while elution with PE10 gave mainly diene esters (not reported). The polyunsaturated esters were not recovered.

<u>Fraction</u>	<u>Weight (g)</u>	<u>% of ML</u>	<u>Composition (by GLC^a)</u>
1	0.18	1.63	no glc peaks, probably hydrocarbons
2	0.44	4.00	mainly P ₁ -P ₃ , no furans
3	0.67	6.09	P ₁ -P ₃ and F ₁ -F ₇
4	0.23	2.09	P ₁ -P ₃ and F ₁ -F ₇
5	0.18	1.63	F ₁ -F ₆ (small) and U ₁ ^b , U ₂ ^b (large)
6	0.05	0.45	mainly U ₁ (also 18:1 ?)
7	trace	-	mainly U ₁ (also 18:1 ?)

^a complete GLC analysis follows

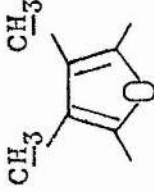
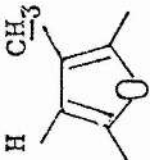
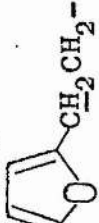
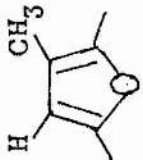
^b U₁ and U₂ are unidentified components of ECL 18.7 and 20.7 respectively.

(i) GLC* (DEGS) of fractions 2-5

<u>Fraction:</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>
<u>Identification</u>	-	-	12.64(0.4)	13.37(0.7)
P ₁₆	14.21(17.8)	14.23(9.5)	14.23(3.1)	14.23(3.1)
?	-	15.30(0.7)	15.64(0.9)	15.47(1.4)
P ₁₉	15.87(20.6)	15.88(4.5)	-	-
?	-	-	16.37(1.4)	16.50(0.6)
?	-	-	-	16.62(1.7)
P ₂₀	17.01(55.7)	17.08(14.7)	16.97(3.1)	16.97(2.9)
16:2?	-	-	17.74(0.7)	17.66(0.8)
16:3?	-	-	18.15(0.9)	18.44(2.0)
U ₁	-	18.83(0.3)	18.77(4.9)	18.74(47.1)
18:2?	-	-	19.35(0.6)	19.29(2.2)
18.3(<u>n</u> -6)?	-	-	19.60(1.6)	19.60(2.2)
?	-	19.93(0.3)	19.88(1.2)	-
F ₁	-	20.30(1.5)	20.30(5.2)	20.30(2.9)

(ii) (a) PMR Spectroscopy of Fractions 2-5

Chemical Shift (δ)

Fraction:	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>
<u>Assignment</u>					
CH_3CH_2-			0.83-0.96 (t)	0.90(t)	
$\begin{array}{c} \text{CH}_3 \\ \\ -\text{CH}- \end{array}$		0.82-0.94 (group of five signals)	-	-	0.82-0.94 (group of five signals)
$-\text{CH}_2\text{CH}_2\text{CH}_2-$	1.14-1.6(m)	1.06-1.6(m)	1.28-1.7(m)	1.28-1.7(m)	1.2-1.6(m)
	-	? 1.78(s)	? 1.78(s)	-	-
	-	? 1.84(s)	? 1.84(s)	-	-
$-\text{CH}_2\text{CH}_2\text{CO}_2\text{CH}_3$	1.86-2.5 (complex)	2.20(t)	2.20(t)	2.22(t)	1.9-2.28
$-\text{CH}_2\text{CH}_2-$ 	-	2.42(t)	2.42(t)	-	-
$-\text{CO}_2\text{CH}_3$	3.65 (s)	3.58(s)	3.58(s)	3.60(s)	3.60(s)
	-	?	5.60(s)	-	-
?	-	-	-	1.68(d)	-
?	-	-	-	5.54(s)	-

U ₂	-	-	20.68(3.3)	20.71(6.5)
F ₂	-	21.21(4.8)	21.18(14.7)	21.20(12.0)
F ₃	-	21.75(2.8)	21.75(5.4)	21.80(3.1)
F ₄	-	22.20(12.4)	22.18(16.5)	22.22(3.2)
F ₅	23.06(1.2)	23.09(4.6)	23.05(9.4)	23.10(2.1)
F ₆	23.61(4.7)	23.64(42.0)	23.63(25.7)	23.69(5.5)
F ₇	-	24.09(1.9)	24.08(1.0)	-

* The first figure shows the ECL and the area percentage is given in parenthesis.

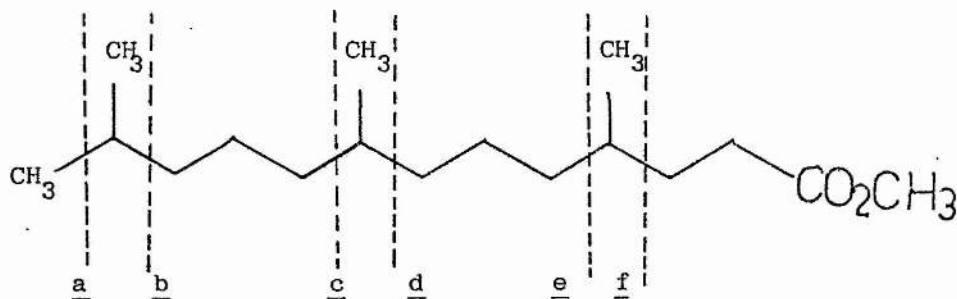
(iii) Gas Chromatography - Mass Spectrometry

The components of fractions 3 and 5 were examined by GC-MS. The mass spectrum of each component peak is tabulated below.

Fraction 3

Peak of ECL 14.0

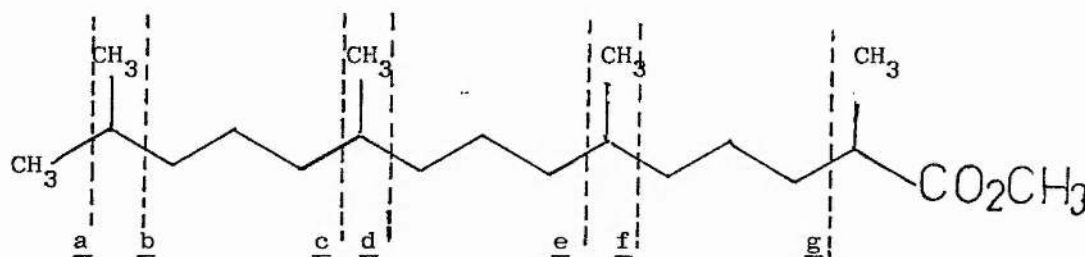
270(M,1), 238(M-32,1), 213(M-57), 157(d,9), 153(c-32,2), 115(e,5), 87(f,100) and 24(43).



The mass spectrum also contained peaks associated with ion fragments $-(CH_2)_nCO_2CH_3$ [101(2)], C_nH_{2n+1} [57(46), 71(28), 85(15), 99(8), 113(6), 127(5), 141(3)], C_nH_{2n-1} [55(33), 69(25), 83(11), 97(6), 111(5), 125(2)], C_nH_{2n-3} [67(2), 81(4), 95(4), 109(2)] and the following additional peaks 197(4), 143(2), 73(6), 59(5).

Peak of ECL 15.8

312(M,7), 222(M-90,5), 157(e,10), 129(f,10), 129(f,5), 125(e-32,3), 88* (g+1,100), and 74(7).

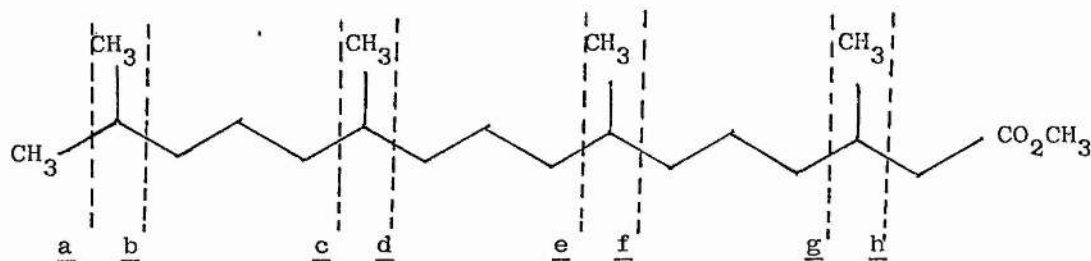


The spectrum also contained peaks associated with ion fragments $-(CH_2)_nCO_2CH_3$ [87(13), 101(39)], C_nH_{2n+1} [57(30), 71(14), 85(6)], C_nH_{2n-1} 55(24), 69(17), 83(8), 97(13), 111(8)], C_nH_{2n-3} [81(3), 95(3)], and the following additional peaks: 143(2), 85(6), 59(6).

Peak of ECL 17.0

326(M,8), 311(a,5), 294(M-32,3), 276(M-50,2), 269(M-57), 213(d,6), 171(e,24), 143(f,14), 139(e-32,26), 121(e-50,7), 101(g,44), 74(h+1,100)*, 69(g-32,38).

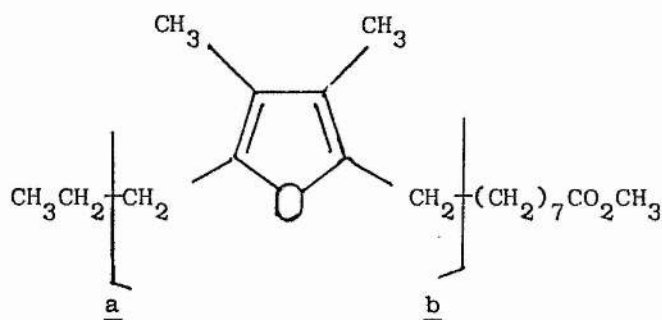
* McLafferty rearrangement ion



The spectrum also contained peaks associated with ion fragments $-(CH_2)_nCO_2CH_3$ [87(80), 115(9), 129(5), 137(10), 151(3), 165(2)], C_nH_{2n+1} [57(45), 71(22), 85(12), 99(6), 113(5), 127(5), 141(3)], C_nH_{2n-1} [55(50), 83(18), 97(18), 111(14), 125(5), 153(4)], C_nH_{2n-3} [67(14), 81(24), 95(14), 109(6), 123(4), 151(3)] and the following additional peaks: 275(6), 222(3), 219(4), 197(6), 186(10), 164(5), 157(14), 135(5), 121(7), 98(20), 93(7), 79(5), 59(16).

Peak of ECL 20.5

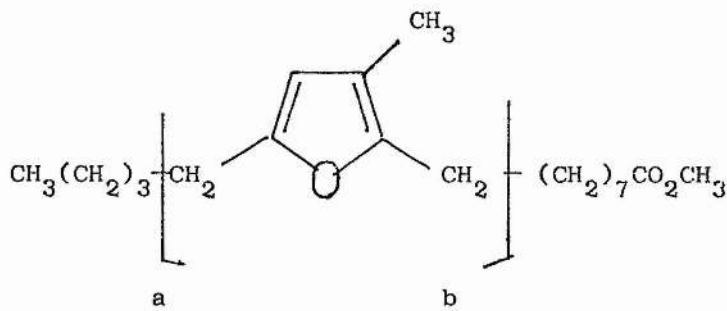
308(M,20), 293(M-15,1), 279(a,23), 277(M-31,5), 165(b+14,4) 151(b,100), 123(a+b,14), and 74(20).



The spectrum also contained peaks associated with ion fragments $-(CH_2)_nCO_2CH_3$ [87(25), 101(25), 115(2), 129(2), 143(3), 157(5), 171(3)], C_nH_{2n+1} [57(19), 71(14), 85(6), 99(3), 113(2)], C_nH_{2n-1} [55(21), 69(14), 83(6), 97(6), 111(4), 125(2), 139(3), 153(8)], C_nH_{2n-3} [67(4), 81(6), 95(6), 109(6), 137(4)], and the following additional peaks: 213(4), 197(3), 177(3), 157(5), 149(6), 135(10), 79(4), 59(4).

Peak of ECL 21.4

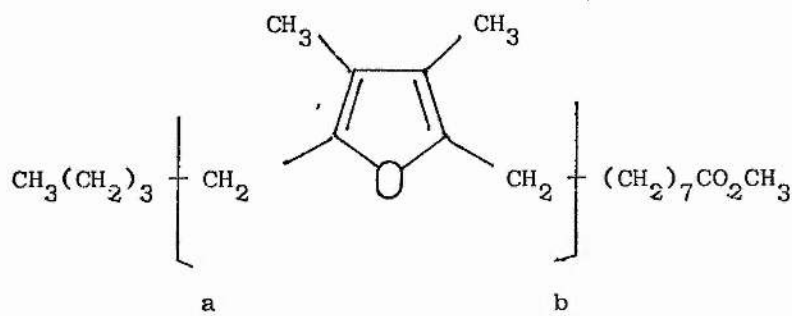
322(M,18), 291(M-31,4), 265(a,7), 179(b+14,2), 165(b,100), 109(a+b,14) and 74(8).



The spectrum also contained peaks associated with ion fragments $-(CH_2)_nCO_2CH_3$ [87(7), 101(8)], C_nH_{2n+1} [57(10), 71(5), 85(2), 99(2)], C_nH_{2n-1} [55(12), 69(6), 83(3), 97(3), 111(2)], C_nH_{2n-3} [67(3), 81(3), 95(1)], and the following additional peaks: 191(2), 163(2), 135(2), 121(7).

Peak of ECL 22.0

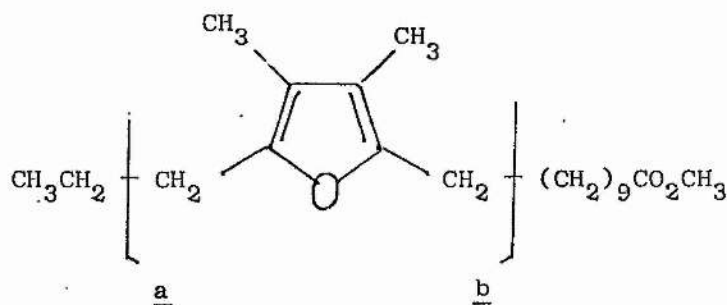
336(M,36), 321(M-15,2), 305(M-31,9), 293(M-43,3) 279(a,41), 193(b+14,4), 179(b,100), 123(a+b,23) and 74(23).



The spectrum also contained peaks associated with ion fragments $-(\text{CH}_2)_n\text{CO}_2\text{CH}_3$ [87(18), 101(20), 115(2), 129(2), 143(2), 157(4), 171(3)], $\text{C}_n\text{H}_{2n+1}$ [57(17), 71(13), 85(7), 99(3), 113(2)], $\text{C}_n\text{H}_{2n-1}$ [55(17), 69(16), 83(7), 97(7), 111(5), 125(2), 139(2), 153(2), 167(2)], $\text{C}_n\text{H}_{2n-1}$ [67(5), 81(4), 95(9), 109(10), 137(11), 151(2), 165(7)], and the following additional peaks: 334(4), 205(4), 149(6), 135(15), 79(4).

Peak of ECL 22.4

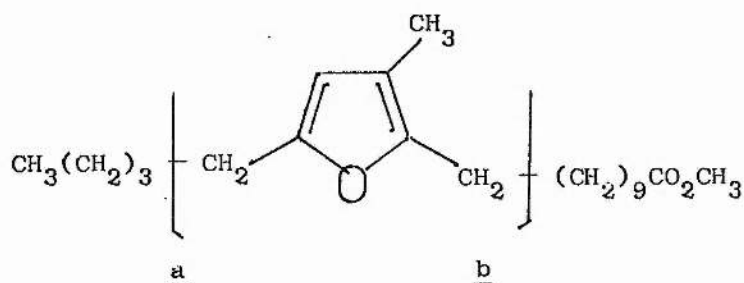
336(M,19), 321(M-15,1), 307(a,19), 305(M-31,5), 293(M-43,1), 165(b+14,5), 151(b,100), 123(a+b,14) and 74(9).



The spectrum also contained peaks associated with fragment ions $-(CH_2)_nCO_2CH_3$ [87(6), 101(9), 115(1), 129(1), 143(1), 157(2)], C_nH_{2n+1} [57(9), 71(5), 85(2), 99(1), 113(1)], C_nH_{2n-1} [55(15), 69(7), 83(3), 97(2), 111(2), 125(1), 139(2), 153(2)], C_nH_{2n-3} [67(3), 81(4), 95(3), 109(6), 137(3)], and the following additional peaks: 326(2), 179(2), 177(3), 149(7), 135(6).

Peak of ECL 23.3

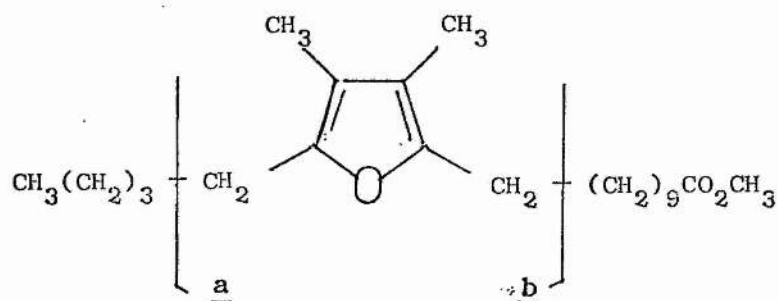
350(M,22), 319(M-31,4), 307(43,3), 293(a,8), 179(b+14,2), 165(b,100), 109(a+b,18), and 74(13).



The spectrum also contained peaks associated with ion fragments $-(CH_2)_nCO_2CH_3$ [87(9), 101(3), 115(1), 129(1), 143(3), 157(2), 171(1)], C_nH_{2n+1} [57(13), 71(5), 85(3), 99(3), 113(1), 127(1)], C_nH_{2n-1} [55(14), 69(10), 83(4), 97(4), 111(3), 125(2)], C_nH_{2n-3} [67(5), 81(5), 95(7), 123(3), 137(2), 151(4)], and the following additional peaks: 219(3), 191(2), 149(2), 135(4), 121(6), 59(3).

Peak of ECL 23.9

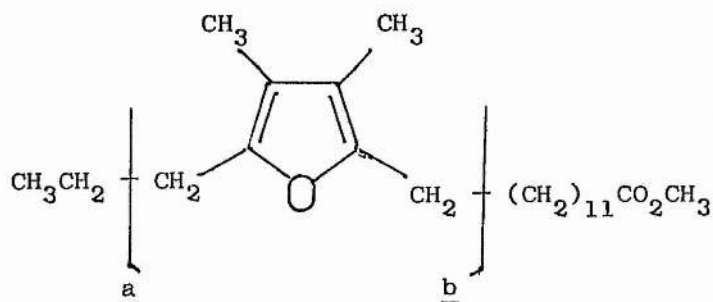
364(M,38), 333(M-31,6), 321(M-43,1), 307(a,31), 193(b+14,4), 179(b,100), 123(a+b,22) and 74(6).



The spectrum also contained peaks associated with fragment ions $-(\text{CH}_2)_n \text{CO}_2 \text{CH}_3$ [87(6), 101(7)], $\text{C}_n \text{H}_{2n+1}$ [57(11), 71(4), 85(2), 99(1), 113(1)], $\text{C}_n \text{H}_{2n-1}$ [55(17), 69(7), 83(3), 97(3), 111(2)], $\text{C}_n \text{H}_{2n-3}$ [67(4), 81(4), 95(3), 109(9), 137(3), 151(2), 165(3)], and the following additional peaks: 204(4), 149(4), 135(10), 122(8), 79(3), 59(3).

Peak of ECL 24.3

364(M, 52), 335(a, 26), 333(M-31, 10), 165(b+14, 9), 151(b, 100), 123(a+b, 19), and 74(20).



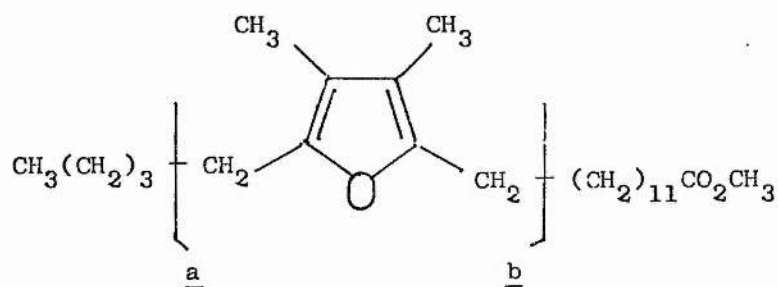
The spectrum also contained peaks associated with ion fragments $-(CH_2)_nCO_2CH_3$ [87(14), 101(27), 115(3), 129(2), 143(4), 157(3), 171(9)], C_nH_{2n+1} [57(19), 71(13), 85(5), 99(4), 113(3), 127(2)], C_nH_{2n-1} [55(21), 69(16), 83(8), 97(6), 111(5), 125(5), 139(3), 153(4), 167(3)], C_nH_{2n-3} [67(6), 81(9), 95(8), 109(8), 137(9)], and the following additional peaks: 360(2), 326(4), 307(14), 291(3), 205(4), 191(3), 182(10), 179(26), 177(11), 157(3), 149(15), 135(12), 122(8), 107(4), 91(3), 79(4), 59(5).

Peak of ECL 25.3

This peak was extremely small in this fraction and the mass spectrum was not recorded.

Peak of ECL 25.7

392(M, 41), 361(M-31, 7), 335(a, 40), 193(b+14, 4), 179(b, 100), 123(a+b, 24) and 74(39).

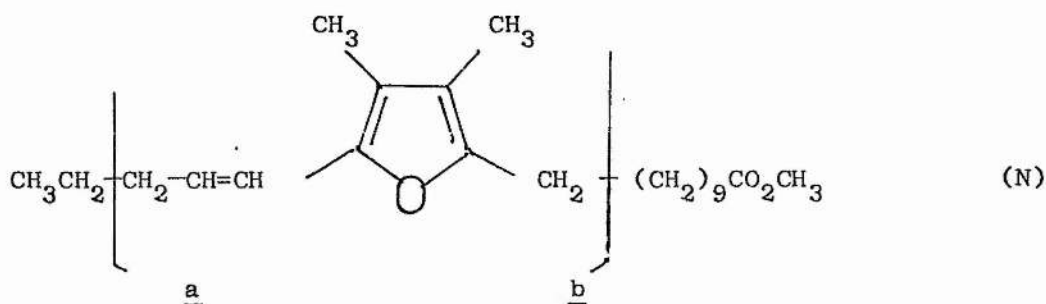
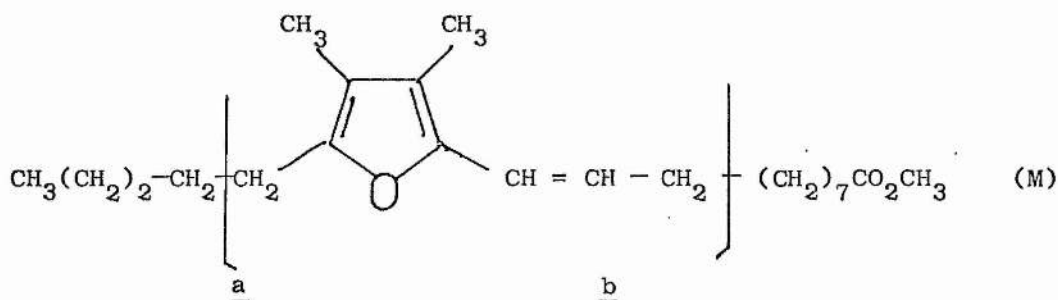


The spectrum also contained peaks associated with fragment ions $-(CH_2)_nCO_2CH_3$ [87(24), 101(59), 115(6), 129(4), 143(5), 157(6)], C_nH_{2n+1} [57(41), 71(22), 85(10), 99(4), 113(3), 127(3)], C_nH_{2n-1} [55(43), 69(29), 83(14), 97(14), 111(10), 125(4), 139(3), 153(3), 167(3), 181(3)], C_nH_{2n-3}

[67(10), 81(8), 95(10), 109(12), 137(5), 151(4), 165(13)],
and the following additional peaks: 362(14), 331(3), 326(3),
312(5), 305(10), 303(3), 205(12), 191(3), 177(25), 176(6),
175(5), 149(44), 135(22), 107(5), 105(5), 93(6), 91(7),
88(30), 79(7), 77(6).

Peak of ECL 26.4

362(M,68), 331(M-31,11)



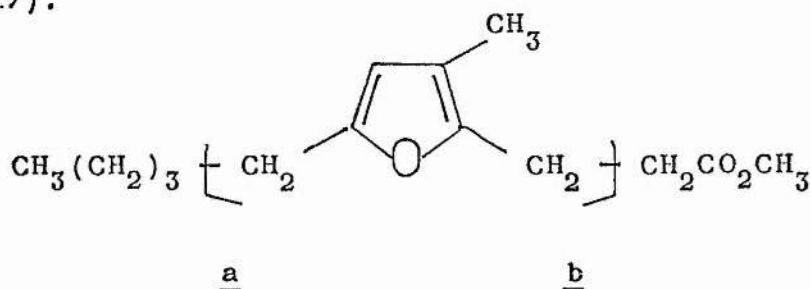
	<u>M</u>	<u>N</u>
<u>a</u>	305(13)	333(39)
<u>b</u>	205(37)	177(79)

The spectrum also contained peaks associated with ion fragments
 $-(CH_2)_nCO_2CH_3$ [87(39), 101(100), 115(11), 143(11), 157(9), 171(13)], C_nH_{2n+1}
 [57(52), 71(31), 85(18), 99(7), 113(6)], C_nH_{2n-1} [55(65), 69(50), 83(23), 97(26),
 111(16), 125(9), C_nH_{2n-3} [67(15), 81(15), 95(21), 109(16), 123(24), 127(11),
 151(16), 165(18), 179(29)]. [74(64)] and the following additional peaks: 360(12),
 326(12), 307(10), 191(9), 175(21), 161(10), 149(18), 135(39), 91(15), 88(42),
 59(12)

Fraction 5

Peak of ECL 15.5

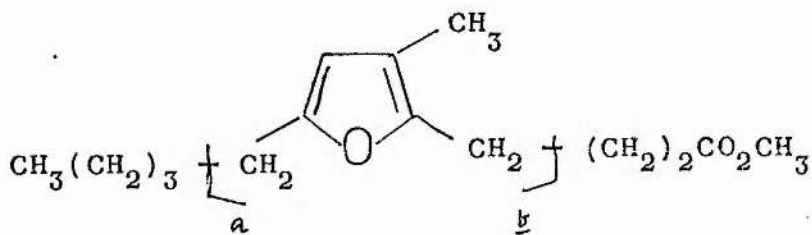
238 (M,24), 181 (a,30), 165 (b,100), 109 (a+b,18),
74 (17).



The spectrum also contained peaks associated with ion fragments $-(CH_2)_n CO_2CH_3$ [87(25)], C_nH_{2n+1} [57(23), 71(10)], C_nH_{2n-1} [55(57), 83(34), 97(15), 111(17)], C_nH_{2n-3} [67(15), 81(18), 95(18)], and the following additional peaks: 121(62), 110(17), 79(10), 77(13).

Peak of ECL 16.6

252 (M,7), 221 (M-31,3), 195 (a,3), 179 (b+14,6),
165 (b,24), 109 (a+b,21), 74 (24).



The spectrum also contained peaks associated with ion fragments $-(CH_2)_n CO_2CH_3$ [87(24)], C_nH_{2n+1} [57(36), 71(12), 85(8)], C_nH_{2n-1} [55(100), 69(63), 83(33), 97(45), 111(12), 125(10), 139(12), 153(5), 167(11)], C_nH_{2n-3} [67(30), 81(36), 95(42), 109(21), 123(14), 137(7), 151(12)], and the following additional peaks: 282(5), 266(4), 234(3), 219(6)

211(4), 194(4), 171(12), 149(6), 135(9), 121(18), 119(7), 115(7), 107(51), 93(30), 91(15), 79(21), 77(11), 59(10).

Peak of ECL 18.7

The mass spectrum of this compound (U_1) contained the following peaks: 294(33), 279(4), 263(5), 245(5), 223(17), 210(8), 195(37), 193(18), 191(4), 179(5), 165(64), 163(27), 149(8), 137(14), 135(16), 123(15), 121(36), 109(14), 107(43), 95(100), 93(37), 91(17), 83(15), 81(40), 79(20), 77(11), 69(21), 67(21), 57(10), and 55(31).

Peak of ECL 20.7

This compound (U_2) showed the following mass spectral peaks: 322(21), 308(6), 307(7), 294(5), 291(5), 251(5), 223(28), 221(6), 210(9), 195(21), 193(32), 179(5), 165(31), 163(13), 155(6), 151(7), 149(6), 137(10), 135(10), 123(15), 121(21), 109(68), 107(36), 95(100), 93(31), 91(14), 83(21), 81(50), 79(17), 69(33), 67(31), 57(16), 55(50).

Peaks at ECL 14.2 and 17.0 were identified as P_{16} and P_{20} respectively, and the ones at 20.3-23.7 as F_1 - F_6 respectively.

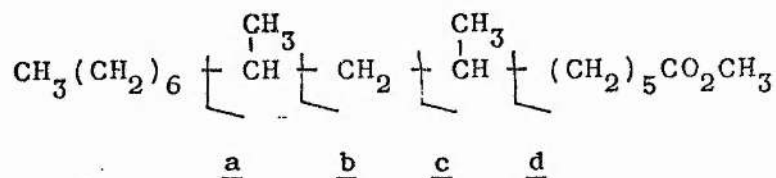
Hydrogenation of Fraction 5

Fraction 5 was submitted to hydrogenation for 20 min. in the presence of Adams catalyst (Platinum dioxide). GLC showed that, under these conditions the furans were virtually unaffected but the peaks of ECL 18.7 (U_1) and 20.7 (U_2) disappeared and two double peaks of ECL 16.3

and 18.3 respectively appeared in their place (see p.50) for complete GLC data). GC-MS of the hydrogenated material gave the following results.

Peaks of ECL 16.3

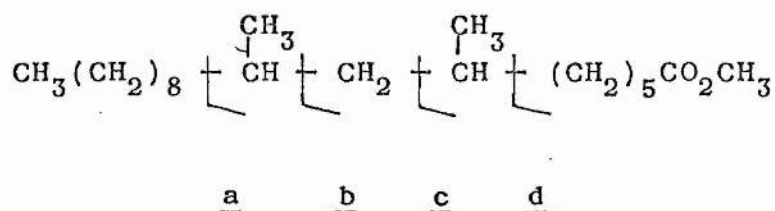
298 (M,10), 283 (M-15,1), 266 (M-32,2), 255 (M-43,2), 199 (a,b), 173 (c+2,2), 172 (c+1,4), 171 (c,5), 167 (a-32,20), 157 (b,43), 149 (a-50,11), 131 (d+2,2), 130 (d+1,10), 129 (d,30) 125 (b-32,31), 107 (b-50,4) and 74 (100).



The spectrum also contained peaks associated with ion fragments $-(\text{CH}_2)_n\text{CO}_2\text{CH}_3$ [87(62), 101(11), 115(7), 143(3)], $\text{C}_n\text{H}_{2n+1}$ [57(57), 71(35), 85(24), 99(6), 113(4)], $\text{C}_n\text{H}_{2n-1}$ [55(52), 69(57), 83(37), 97(33), 111(12), 125(31), 139(13)], $\text{C}_n\text{H}_{2n-3}$ [67(10), 81(11), 95(10), 109(9), 123(6), 137(13)], and the following additional peaks: 248(6), 185(7), 165(10).

Peak of ECL 18.3

326 (M,28), 295 (M-31,4), 199 (a,13), 173 (b+2,3), 172 (b+1,5), 171 (b,11), 167 (a-32,21), 157 (c,34), 149 (a-50,13) 131 (d+2,6), 130 (d+1,17), 129 (d,28), 125 (c-32,21), 107 (c-50,4), and 74 (100).



The mass spectrum also contained peaks associated with ion fragments $-(\text{CH}_2)_n \text{CO}_2 \text{CH}_3$ [87(79), 101(17), 115(13), 143(21), 185(16)], $\text{C}_n \text{H}_{2n+1}$ [55(69), 69(72), 83(48), 97(38), 111(24), 139(11), 153(16), 181(4), 195(13)], $\text{C}_n \text{H}_{2n-1}$ [57(79), 71(48), 85(31), 99(11), 113(7), 127(6), 141(12), 155(13)], $\text{C}_n \text{H}_{2n-3}$ [67(21), 81(17), 95(21), 109(10), 123(11), 137(11), 151(3), 165(21)].

The 9,11-Dimethyl 18:0 structure is also possible for this data (See discussion).

Investigation of *Exocarpus cupressiformis* seed oil

Seed were harvested from road side habitats in Sydney, Australia (supplied by CSIRO). Sample A contained seed from more than one shrub and sample B was from a single tree.

Seed oils were transesterified with methanol by both acid (sulphuric) and base (sodium methoxide) catalysis. Total esters were examined by GLC (DEGS and SP 2300) before and after silylation. Sample A was analysed in greater detail by the following additional operations.

The methyl esters (4.0 g) and urea (20 g) were crystallized from methanol (120 ml) to obtain a colourless adduct (2.83 g) and a dark yellow liquor (0.23 g). A gum which separated during this operation was discarded.

Prep. TLC of the mother liquor gave fractions: A (18 mg, 11%), B (31 mg, 19%), C (97 mg, 61%) and D (13 mg, 9%).

Fraction C

GLC (SP 2300, 240⁰) of this fraction showed a single peak of ECL 27.1 which was converted to a peak of ECL 22.3 after silylation. The IR spectrum of C showed peaks at 3430 (b,s,-OH), 2200 (f,w,-C≡C-), and 950 cm⁻¹ (f,s,-CH^t=CH-C≡C-). Its UV spectrum had maximum absorption at 229 nm (ε = 12,400, hexane).

The PMR spectrum of C contained signals at 0.88 (t, 3H, CH₃CH₂-); 1.30-1.7 (m, H,-CH₂CH₂CH₂-); ~ 2.1 (multiplet -CH₂CH=CH-), and 2.24 (t, -CH₂CH₂CO₂CH₃) (altogether 4H);

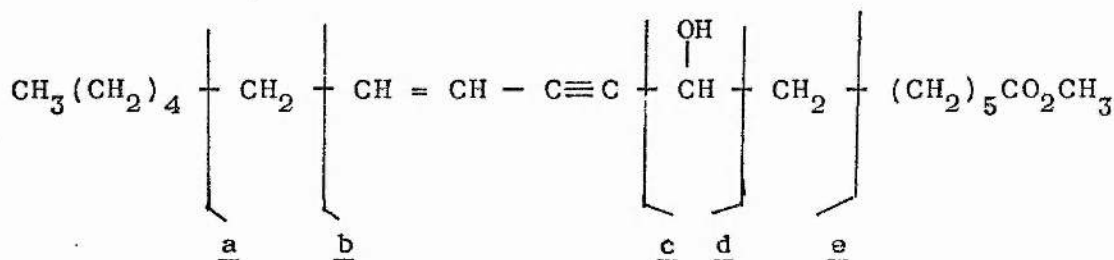
2.55 (m, 2H, $-\text{C}\equiv\text{CCHCH}_2\text{CH}_2-\text{?}$); 3.60 (s, 3H, $-\text{CO}_2\text{CH}_3$); 4.35

(m, 1H, $-\text{C}\equiv\text{CCHCH}_2-$); 5.42 (d, $J = 16\text{ Hz}$, 1H, $-\text{CH}_2-\text{C}=\text{C}-\text{H}$)

and 6.07 (a doublet, $J = 16\text{ Hz}$, each of which was split into

triplets, $J = 7\text{ Hz}$, $-\text{CH}_2-\text{C}=\text{C}-\text{H}$).

The mass spectrum of C displayed peaks at 223 (b, 3), 205 (a-32, 2), 180 (e+1, 5), 173 (c, 6), 165 (d, 57), 147 (d-18, 5), 141 (c-32, 9), 123 (c-50, 7), 95 (a+d+1, 100), 81 (b+d+1, 100), and 74 (18).



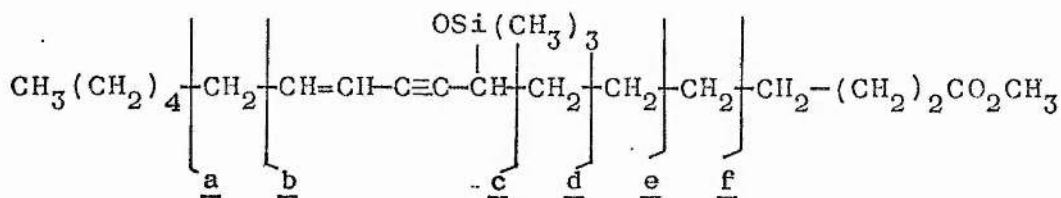
It also contained peaks associated with ion fragments $-(\text{CH}_2)_n\text{CO}_2\text{CH}_3$ 87(100), 101(27), 115(6), $\text{C}_n\text{H}_{2n+1}$ 57(20), 71(10), 85(6), $\text{C}_n\text{H}_{2n-1}$ 55(87), 69(50), 83(19), 97(10), 111(7), $\text{C}_n\text{H}_{2n-3}$ 67(60), 109(23), and the following additional peaks: 291(1), 233(1), 218(5), 203(3), 163(6), 149(4), 144(9), 119(14), 107(20), 105(24), 93(42), 91(50), 79(50), 76(19), 65(15), 59(19).

The major peaks in the CMR spectrum of C were as follows: 145.40(50), 138.79(26), 108.95(53), 62.96(74), 51.40(41), 37.92(76), 37.69(23), 34.10(75), 33.07(66), 32.32(26), 31.67(64), 29.07(99), 28.95(120), 28.77(111),

28.35(24), 28.29(22), 25.45(20), 25.02(89), 24.92(99), 22.59(55), and 14.04(45).

Silylation of fraction C

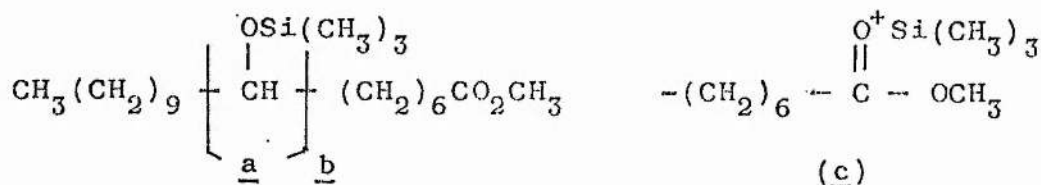
Fraction C was submitted to silylation and the mass spectrum of the product displayed peaks at 380 (M,0.2), 365 (M-15,2), 349 (M-31,0.5), 309 (a,1), 295 (b,4), 279 (f,2), 265 (e,2), 251 (d,2), 237 (c,100), 75 [HO = Si(CH₃)₂,40], 74 (14), 73 [Si(CH₃)₃, 100].



The mass spectrum also contained peaks associated with ion fragments C_nH_{2n+1} 57(6), C_nH_{2n-1} 55(17), 69(10), C_nH_{2n-3} 67(9), 81(6), 95(6), and the following additional peaks: 218(3), 211(3), 207(3), 193(4), 181(2), 169(4), 168(4), 167(7), 149(7), 129(3), 119(3), 117(3), 105(7), 93(5), 91(12), 89(5), 79(7), 78(5), 77(5), 65(4), 61(3), 59(12).

Hydrogenation of fraction C

Hydrogenation of C, produced GLC peaks of ECL 24.7 (78%) and 18.0 (22%). The hydrogenation product was submitted to silylation and mass spectrum of the resulting material displayed peaks at 245 (a,23), 243 (b,13), 216 (c,2), 213 (a-32,1), 153 (b-90,9), 75 [HO = Si(CH₃)₂,46], and 73 [Si(CH₃)₃,59].



Fraction D

This was a crystalline material which did not show peaks on GLC (SP 2300) and was not examined further.

Fraction A

GLC of fraction A showed peaks of ECL 19.8 (4%), 20.4 (14%), 21.9 (18%), 24.1 (49%), and 24.5 (15%). Apart from a weak signal at 950 cm^{-1} , its IR spectrum did not show any unusual absorptions and this fraction was not investigated further.

Fraction B

Except for a weak signal at 950 cm^{-1} , this substance (ECL 24.3) did not show any unusual absorptions in the IR. Its UV spectrum, however, exhibited absorptions at 214, 227, 237, 253, 267 and 281.

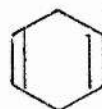
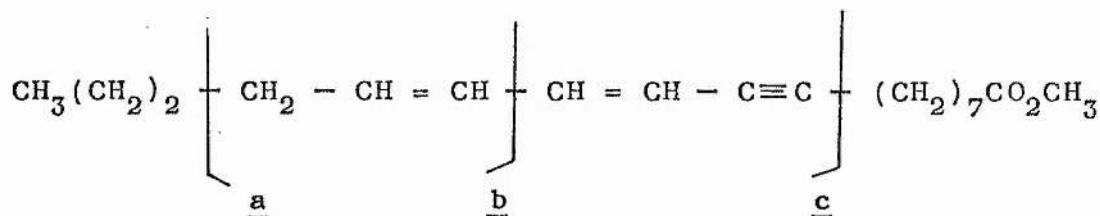
Silica Acid Chromatography of the Urea Adduct

Since the substance of ECL 24.3 (fraction B) was concentrated in the urea adduct, the adduct (2.30 g) was separated by column chromatography (Sorbsil, 100 g), with the following results.

<u>Fraction No</u> ^a	<u>Eluting Solvent</u>	<u>Weight</u>	<u>Composition (by GLC)</u>
1	PE5	-	-
2	PE5	-	-
3	PE5	1.896	<u>b</u>
4	PE5	0.012	21.9 (87%), 24.3 (13%)
5	PE5]	0.087	21.9 (23%), 24.3 (77%)
6	PE5]		
7	PE5	-	-
8	PE50]	0.014	21.9 (6%), 23.2 (94%)
9	PE50]		

- a 100 ml fractions were collected
- b Apart from the absence of the components of ECL 23.2 and 24.3, this fraction was similar in composition to that of the total urea adduct
- c Attempts to purify this combined fraction by Ag^+ TLC (PE20) were unsuccessful.

The mass spectrum of the combined fraction 5,6 displayed peaks at 290 ($\text{M}, 0.1$), 259 ($\text{M}-31, 2$), 133 (c, 21), 91 (a+c+1, 70), 83 (b, 100), 79 (d?, 70).



d

It also contained peaks associated with ion fragments $-(\text{CH}_2)_n \text{CO}_2 \text{CH}_3$ 87(12), $\text{C}_n \text{H}_{2n+1}$ 57(9), 71(3), 85(3), $\text{C}_n \text{H}_{2n-1}$ 55(66), 69(31), 97(4), $\text{C}_n \text{H}_{2n-3}$ 67(66), 81(47), 95(23), 109(6) 74(28).
the following additional peaks: 159(3), 150(11), 147(8), 131(13), 119(45), 117(13), 107(19), 105(43), 93(52), 80(59), 78(40), 59(22).

EXPERIMENTAL: Section II - SYNTHESIS

I. PREPARATION OF STARTING MATERIALS

1. Methyl cis-12,13-epoxyoctadec-cis-9-enoate

Cephalocroton cordofanus seed oil (5 g) was transesterified with sodium methoxide in methanol (0.5 M, 30 ml) at room temperature, overnight. Methyl cis-12,13-epoxyoctadec-cis-9-enoate was isolated by column chromatography (Sorbil, 120 g) of the total esters. The column was eluted first with PE3 to remove small quantities of hydrocarbons and other non polar material. Elution with PE8 furnished the pure epoxy-ester [2.7 g, ECL 23.7 (SP 2300)].

Spectra data:

PMR signals at 0.90 (t, 3H, CH_3CH_2-); 1.26-1.6 (m, 20H, $-\text{CH}_2\text{CH}_2\text{CH}_2-$); 1.9-2.1 (m, 2H, $-\text{CH}-\text{CH}(\text{O})\text{CH}_2\text{CH}=\text{CH}-$)
 2.20 (t, 2H, $-\text{CH}_2\text{CH}_2\text{CO}_2\text{CH}_3$); 2.72 (m, 2H, $-\text{CH}_2\text{CH}(\text{O})\text{CHCH}_2-$)
 3.58 (s, 3H, $-\text{CO}_2\text{CH}_3$); and 5.4 (m, 2H, $-\text{CH}_2\text{CH}=\text{CHCH}_2-$).

CMR signals at: 173.89(6), 132.48(43), 124.25(40), 57.20(37), 56.42(39), 51.24(23), 34.80(39), 31.89(36), 29.23(120), 27.94(44), 27.52(47), 26.44(79), 25.05(43), 22.72(42), 14.02(34).

2. Methyl threo-12,13-dihydroxyoctadec-cis-9-enoate, ³²⁹

Vernonia anthelmintica seed oil (50 g) was boiled with glacial acetic acid (350 ml) for 7 hrs. The product was then hydrolysed by boiling with 1 M alcoholic potassium hydroxide (300 ml) for 1 hr. Most of the alcohol was distilled off under reduced pressure, the product diluted with water, and the unsaponifiable material removed by extraction with ether. Acidification of the aqueous layer and extraction with ether furnished the impure dihydroxy acid. The pure acid (m.p. 52-54°) was obtained by crystallization from petrol (b.p. 40-60°). The methyl ester was prepared with methanolic sulphuric acid (2%). [ECL 21.6 bis(OTMS), (DEGS)]. Its IR spectrum contained peak at 3420 (b, s -O-H), and 3000 cm⁻¹ (f, m), -C=C-H).

3. Methyl trans-11,12-epoxyoctadec-9-ynoate

(i) Preparation of methyl octadec-trans-11-en-9-ynoate

The mixed acids of Santalum album seed oil (2 g) were crystallized several times from petrol to yield pure octadec-trans-11-en-9-ynoic acid (Xymenynic acid, 1.4 g, m.p. 38°). Methylation of the acid with methanolic boron trifluoride afforded the ester. The PMR spectrum of Xymenynic acid showed signals at 0.88 (t, CH₃CH₂-); 1.28-1.7 (m, -CH₂CH₂CH₂-); 2.0-2.2 (broad triplet, -C≡C-CH₂CH₂-); 2.30 (t, -CH₂CH₂CO₂CH₃), 5.5 (m, -CH₂-CH^t=CH-C≡C-); and ~5.9 (m, -CH₂-CH^t=CH-C≡C).

Its IR spectrum contained peaks at 2200 (f, w, -C≡C-) and 950 cm⁻¹ (f, s, -CH=CH^t-C≡C-). The CMR spectrum contained the following signals: 173.38(6), 142.48(28), 109.71(28),

87.90(12), 78.95(8), 65.33(6), 50.73(19), 33.56(34), 32.53(34), 32.14(3), 31.30(28), 29.95(5), 29.65(2), 29.29(3), 28.60(54), 28.41(120), 26.96(4), 24.51(32), 22.18(25), 18.90(33), 14.80(8), and 13.56(20).

(ii) Epoxidation of methyl octadec-trans-11-en-9-ynoate

A mixture of methyl octadec-trans-11-en-9-ynoate (1.00 g, 2.93 mmol) and *m*-chloroperbenzoic acid (87%, 0.60 g) in methylene chloride solution was stirred for 2 h, and allowed to stand at room temperature, overnight. The solvent was removed under reduced pressure and the residue was treated with a saturated solution of sodium bisulphite (25 ml) to destroy any excess peracid. The organic product was extracted with diethyl ether (2 x 50 ml), washed first with aqueous sodium bicarbonate (10%, 2 x 25 ml), and then with brine solution (5%, 25 ml)*. Purification of the product (0.98 g) by column chromatography (Sorbil, 30 g, using PEO-P38 as eluting solvent) gave methyl trans-11,12-epoxyoctadec-9-ynoate, [0.87 g, ECL 25.0 (SP 2300)].

Spectral Data:

PMR signals at 0.88 (t, 3H, CH_3CH_2-), 1.3-1.7 (m, 20H, $-\text{CH}_2\text{CH}_2\text{CH}_2-$), 2.08-2.30 (two overlapping triplets, 4H, $-\text{CH}_2\text{CH}_2\text{CO}_2\text{CH}_3$ and $-\text{C}\equiv\text{CCH}_2\text{CH}_2-$), 2.86 (m, 2H, $-\text{CH}_2\text{CH}(\text{O})-\text{CHC}\equiv\text{C}-$), and 3.56 (s, 3H, $-\text{CO}_2\text{CH}_3$). Its IR spectrum contained peaks at 2210 (f,w, $-\text{C}\equiv\text{C}-$) and 870 cm^{-1} (f,s, $-\text{CH}(\text{O})-\text{CH}-$).

* In subsequent epoxidations this method will be referred to as the "usual procedure".

4. Methyl cis-9,10-epoxyoctadec-12-ynoate

This was prepared by epoxidation of methyl octadec-cis-9-en-12-ynoate (methyl crepenynate), isolated from Afzelia cuanensis* seed oil as follows.

The seed oil methyl esters (80 g) and urea (375 g) were crystallized from methanol (2.2 l) at 0°, overnight. This removed all of dehydrocrepenynate (18:3 9c12a14c) [ECL 21.7 (DEGS)], which was retained in the mother liquor (19 g) with small amounts of methyl linoleate. Methyl esters recovered from the adduct (52 g) were crystallized once more with urea (100 g) from methanol (600 ml). This time, the mother liquor (23 g) contained methyl crepenynate, methyl linoleate and a small quantity of methyl oleate. A portion of it (3 g) was purified by argen^tation column chromatography [Sorbisil (120 g), containing 30% silver nitrate]. Elution with PE5-PE20 furnished a concentrate (2.7 g) composed of methyl crepenynate [54%, ECL 21.1 (DEGS)] and methyl linoleate [46%, ECL 19.2 (DEGS)].

Epoxidation

Methyl crepenynate concentrate (300 mg) [containing, 18:2(9c12a) (0.66 mmol) and 18:2(9c12c) (0.47 mmol)] and *m*-chloroperbenzoic acid (87%, 350 mg, 1.76 mmol) were stirred for 2 hr in methylene chloride (15 ml) solution and allowed to stand overnight. The product (314 mg) was recovered in the usual manner, and purified by Prep. TLC (PE30).

* Contained 18:2(9c12c) 26%, 18:2(9c12a) 28%, and 18:3(9c12a14c) 17%.

[132 mg, BCL 24.7 (SP 2300), and 25.2 (DEGS)].

Spectral Data:

PMR signals at 0.90 (t, 3H, CH_3CH_2-); 1.2-1.7 (m, 20H, $-\text{CH}_2\text{CH}_2\text{CH}_2-$); two overlapping triplets (4H) at 2.10 ($-\text{C}\equiv\text{CCH}_2\text{CH}_2-$) and 2.22 ($-\text{CH}_2\text{CH}_2\text{CO}_2\text{CH}_3$); 2.6-3.0

(m, 2H, $-\text{CH}_2\overset{\text{O}}{\underset{\text{O}}{\text{CH}}}-\text{CHCH}_2-$); and 3.58 (s, 3H, $-\text{CO}_2\text{CH}_3$).

5. Methyl 12-hydroxyoctadec-9-ynoate.

[BCL 25.5 (DEGS)] was available in the laboratory.

6. Methyl 12-hydroxyoctadec-cis-9-enoate

Castor oil methyl esters (10 g, containing 87% methyl 12-hydroxyoctadec-cis-9-enoate) was purified by column chromatography (Sorbisil, 250 g). Elution with PE10 removed all of the non-oxygenated esters and PE25 furnished the pure ester required [7.6 g, BCL 24.1 (SP 2300), 24.8 (DEGS)].

7. Methyl 9-hydroxyoctadec-cis-12-enoate

This was isolated from Strophanthus courmontii seed oil by the same method outlined for the preparation of methyl 12-hydroxyoctadec-cis-9-enoate [BCL 24.1 (SP 2300), 25.1 (DEGS)].

8. Methyl 12-oxooctadec-cis-9-enoate ³³⁰

Methyl 12-hydroxyoctadec-cis-9-enoate (5 g) in glacial acetic acid (50 ml) was oxidized by addition, all at once, of a solution of sodium dichromate dihydrate (3.2 g)

in water (4.0 ml), acetic acid (30 ml), and concentrated sulphuric acid (1.8 ml). After stirring vigorously for 30 seconds, the solution was diluted with iced-water (125 ml), and the organic product (4.9 g) isolated by extraction with ether. The crude product was purified by chromatography (Sorbisil 120 g, PE10) [ECL 24.5 (SP 2300)].

Spectral Data:

PMR signals at 0.88 (s, 3H, CH_3CH_2-); 1.2-1.7 (m, 18H, $-\text{CH}_2\text{CH}_2\text{CH}_2-$); 1.9-2.1 (m, 2H, $-\text{CH}=\text{CH}-\text{CH}_2\text{CH}_2-$); two partially overlapping triplets at 2.20 (2H, $-\text{CH}_2\text{CH}_2\text{CO}_2\text{CH}_3$), and 2.34 (2H, $-\text{CH}_2\text{CH}_2\text{CO}-$); 3.02 (d, 2H, $-\text{COCH}_2\text{CH}=\text{CH}-$); 3.58 (s, 2H, $-\text{CH}_2\text{CH}=\text{CHCH}_2-$). Its IR spectrum contained peaks at 3010 (f,w, $-\text{C}=\text{C}-\text{H}$) and 1715 cm^{-1} (f,s, $-\text{CO}-$).

9. Methyl 12-hydroxyoctadec-trans-9-enoate³³¹

A mixture of 12-hydroxyoctadec-cis-9-enoate (628 mg, 2.00 mmol) and 3-mercaptopropionic acid (424 mg, 4.00 mmol) in ethanol (25 ml) was allowed to stand at room temperature for 3 days. The product, containing both cis and trans isomers, was separated by Ag^+ TLC (PE30), to obtain the pure trans-isomer (338 mg).

Its IR spectrum contained peaks at 3430 (b,s, $-\text{O}-\text{H}-$), 3010 (f,w, $-\text{C}=\text{C}-\text{H}$), and 970 (f,s, $-\text{C}=\text{C}-$).

10 Methyl cis-9,10; cis-12,13-diepoxyoctadecanoate

Methyl octadeca-cis-9,cis-12-dienoate (2.0 g, 6.80 mmol) and m-chloroperbenzoic acid (87%, 2.69 g, 13.60 mmol) were stirred for 2 h in methylene chloride (100 ml) at room temperature, and allowed to stand overnight. The

product (1.9 g) was isolated according to the usual procedure and purified by column chromatography (Sorbisil 50 g). The pure diepoxide (0.91 g) was obtained by elution with PE20 and its GLC (SP 2300) showed two peaks of ECL 27.8 (63.4%) and 28.5 (36.6%).

Spectral Data:

The PMR spectrum of the diepoxide contained signals at 0.91 (t, 3H, CH_3CH_2-); a multiplet at 1.4 ($-\text{CH}_2\text{CH}_2\text{CH}_2-$); and a triplet overlapping with it at 1.61

($-\text{CH}-\text{CHCH}_2\text{CH}-\text{CH}-$); 2.24 (t, 2H, $-\text{CH}_2\text{CH}_2\text{CO}_2\text{CH}_3$); 2.80-3.04 (m, 4H, $-\text{CH}-\text{CH}-\text{CH}_2\text{CH}-\text{CH}-$); and 3.60 (s, 3H, $-\text{CO}_2\text{CH}_3$).

Its IR spectrum contained peaks at 820 and 840 cm^{-1} (f,w), $-\text{CH}-\text{CH}-$

11. Diepoxides of methyl octadeca-cis-9,cis-12,cis-15-trienoate

Methyl octadeca-cis-9,cis-12,cis-15-trienoate (99%), 5.00 g, 17.1 mmol) and m-chloroperbenzoic acid (87%, 6.77 g, 34.2 mmol) were stirred in methylene chloride (100 ml) at room temperature, for 3 h, and allowed to stand overnight. The crude product (5.53 g) recovered in the usual way was purified by column chromatography (Sorbisil, 125 g). The mono-epoxides (1.23 g) were eluted with PE8 and the diepoxides (2.86 g) with PE20. Finally, elution with PE40 furnished some triepoxides (0.62 g). GLC (SP 2300, 250°) of the diepoxides showed three peaks of ECL 28.0 (5%), 28.5 (31%), and 29.3 (64%).

11. Methyl octadeca-trans-8,trans-10-dienoate

This compound was prepared by bromination-debromination of oleic acid, according to the following procedure.³³²

(a) Allylic bromination

A mixture of oleic acid (>99%, 10.0 g, 0.034 mol), carbon tetrachloride (65 ml), N-bromosuccinimide (freshly recrystallized from water, 13.32 g, 0.074 mol) and benzoyl peroxide (11 mg) (added in that order) was refluxed for 12 h, and kept at 0°, overnight. It was then filtered (to remove the succinimide) and the solvent removed to produce a dark brown oil (15.12 g).

(b) Bromination of the double bond

Bromine (5.5 g, 0.034 mol) was added dropwise to a stirred solution of the product (a) in ether (75 ml), the temperature being maintained between -10 and 0°C. The reaction mixture was then allowed to warm to room temperature, and the solvent removed to obtain a dark brown product (17.20 g).

(c) Debromination³³³

Zinc powder (17 g) was added to a solution of the tetrabromide (11b) in ethanol (50 ml). The reaction mixture was warmed slightly (CAUTION: Vigorous reaction!) and finally refluxed for 30 min. under nitrogen. The product (15.24 g) contained both acids and esters (TLC) and was ^asaponified with alcoholic potassium hydroxide (1 M, 25 ml).

(d) Isomerization

The acids (11c) were taken up in petrol (15 ml) and irradiated with ultraviolet light (tungsten lamp, 150 w) for 4 h in the presence of a trace of iodine. The petrol solution was washed with dilute sodium thiosulphate and dried. Crystallization at -20° , overnight, furnished the crude conjugated diene (11.72 g). Two successive recrystallizations from petrol at -20° afforded colourless crystals (5.71 g, m.p. $37-38^{\circ}$). GLC of the methyl esters (SP 222) showed peaks of ECL 20.3 (4%) and 21.0 (96%).

Spectral Data:

The PMR spectrum contained signals at 0.89 (t, 3H, CH_3CH_2-); 1.28-1.65 (m, $-\text{CH}_2\text{CH}_2\text{CH}_2-$); two partially overlapping signals (6H) at 2.03 (q, $-\text{CH}_2\text{CH}_2\text{CH}=\text{CH}-$), and 2.22 (t, $-\text{CH}_2\text{CH}_2\text{CO}_2\text{CH}_3$); 3.58 (s, 3H, $-\text{CO}_2\text{CH}_3$); 5.29-5.57 (m, 2H, $-\text{CH}_2\text{CH}=\text{CHCH}=\text{CHCH}_2-$); and 5.75-6.05 (m, 2H, $-\text{CH}=\text{CHCH}=\text{CH}-$). Its IR spectrum showed peaks at 3010 (f,s, $-\text{C}=\text{C}-\text{H}$), 1620 (f,w, $-\text{C}=\text{C}-$), and 990 cm^{-1} (f,s, $-\text{CH}=\text{CH}-\text{CH}=\text{CH}-$). In addition to a maximum at 231 nm ($\epsilon = 29, 870$, hexane), its UV spectrum contained inflexions at 224 and 237. The major peaks in the CMR spectrum were as follows: 174.11(11), 132.46(36), 132.12(12), 131.98(42), 130.61(36), 130.39(39), 51.32(41), 34.08(57), 32.90(10), 32.62(54), 32.53(46), 31.89(45), 29.49(60), 29.24(120), 29.06(70), 28.82(47), 24.95(50), 22.69(41), and 14.07(44).

II. SYNTHESSES INVOLVING PALLADIUM (II) CATALYSIS

1. Methyl 12,13-epoxyoctadec-cis-9-enoate

Methyl 12,13-epoxyoctadec-cis-9-enoate (620 mg, 2.00 mmol), palladium (II) chloride (18 mg, 0.10 mmol), and cupric chloride (dehydrated by heating at 110° for 2 h, 267 mg, 2.00 mmol) were reacted in dimethylformamide according to the following general procedure*.

The reactants were contained in a two-necked flask (100 ml) and agitated by a stream of air (dried by passage through silica gel) drawn through the reaction mixture. The flask was heated on a steam bath until GLC showed that all the epoxy ester had reacted (18 h in this reaction). The reaction mixture was then cooled and diluted with water (30 ml). The organic product was extracted with ether (2 x 25 ml) and the combined ether extracts filtered (to remove any suspended palladium), washed with water, and dried over anhydrous sodium sulphate. Finally, removal of the solvent in a rotary film evaporator furnished the reaction product (607 mg).

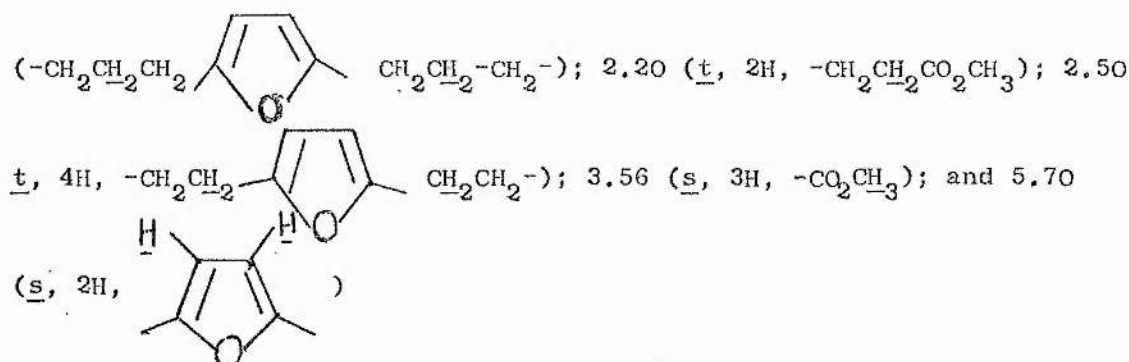
GLC examination (DEGS, 185°) of the product showed peaks of ECL 21.2 (81%), 21.6 (3%), 24.9 (10%), and 25.8 (6%), (some small peaks and a major late eluting peak were ignored but will be discussed later).

* In the following sections this method will be referred to as "the usual procedure".

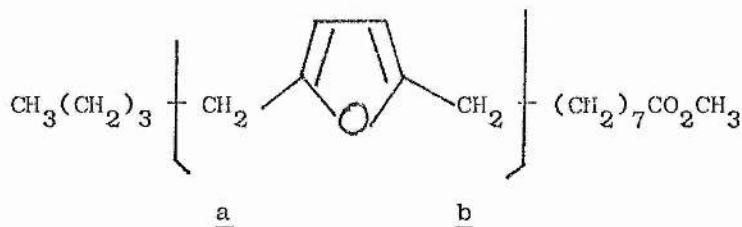
A portion of the product (450 mg) was separated by Prep. TLC (PE20) giving fractions A (180 mg, 52%), B (40 mg, 12%) and C (125 mg, 36%).

Fraction A

This compound of ECL 21.2 had a R_f value (TLC) similar to that of an authentic long-chain furanoid ester. Its UV spectrum showed an absorption maximum at 222 nm ($E_{\text{max}} = 9,500$, hexane). The IR spectrum had absorption peaks at 3100 (f,w), 1610 (f,w), 1560 (f,s), 1010 (f,s) 950 (f,m), and 775 cm^{-1} (f,s), in addition to the signals usually associated with fatty esters. The PMR spectrum contained signals at 0.88 (t, 3H, CH_3CH_2^-); multiplets (total of 16H) at 1.30 ($-\text{CH}_2\text{CH}_2\text{CH}_2^-$) and 1.58



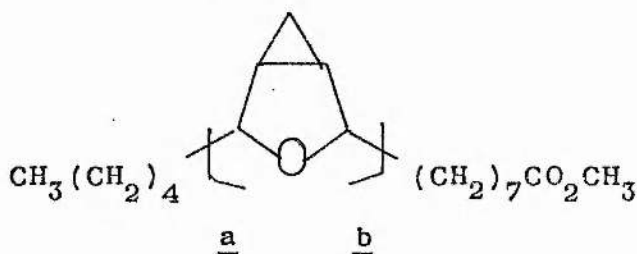
Its mass spectrum showed peaks at 308 (M,8), 277 (M-31,3), 251 (a,7), 165 (b+14,13), 151 (b,100), 95 (a+b,64), 81 (c,23), 74 (31), and 69 (d,28).



It also showed peaks associated with ion fragments, $-(CH_2)_n CO_2 CH_3$ [87(10)], $C_n H_{2n+1}$ [57(46), 71(28), 85(8), 99(11)], $C_n H_{2n-1}$ [55(49), 83(11), 97(8)], $C_n H_{2n-3}$ [67(13), 109(8)], and the following additional peaks: 121(8), 107(12).

Fraction B

GLC of this fraction showed peaks of ECL 21.6 (20%), 24.9 (43%) and 25.8 (37%) accompanied by several minor peaks which were ignored. The chromatograph was unaffected by silylation. The material showed a single spot on TLC, running between fraction A and the starting epoxy ester. Apart from weak absorptions at 3420 (b) and 970 cm^{-1} (f), its IR spectrum did not show any unusual absorptions. The mass spectrum of the component of ECL 21.6, obtained by GC-MS displayed peaks at 311(M+1,2), 310(M,8), 279(M-31,8), 267(M-43,4), 239(a,100), 221(a-18,5), 207(a-32,32), 189(a-50,6), 153(b,9), and 74(10).



It also contained peaks associated with ion fragments $-(CH_2)_n CO_2 CH_3$ [87(6)], $C_n H_{2n+1}$ [57(7), 71(7), 85(6), 99(14), 113(3), 127(6)], $C_n H_{2n-1}$ [55(26), 69(14), 83(16), 97(8), 111(8), 125(5)], $C_n H_{2n-3}$ [67(30), 81(28), 95(20), 109(12), 123(5), 137(2)], and the following additional peaks: 254(7), 171(7), 155(8), 151(16),

147(10), 139(3), 107(10), 105(8), 93(11), 91(7), 79(16), 68(16) and 54(22).

Fraction C

GLC of this fraction showed a major peak of ECL 28.0 which was converted to a peak of ECL 22.9 after silylation. TLC (PE20) showed two or more polar, overlapping spots. Its IR spectrum contained absorption peaks at 3490 (b,m), 1810 (f,s) and 970 cm^{-1} (f,m) in addition to the usual signals. The mass spectrum of the component of ECL 22.9 which was obtained by GC-MS of the silylated product displayed peaks at 382(M,9), 351(M-31,6), $75(\text{HO}-\text{SiMe}_2, 64)$ and $73(\text{SiMe}_2^+, 100)$. It also contained major peaks at 299(28), 270(58), 239(16), 223(42), 221(100), 185(18), 165(7), 129(10), 123(7), 113(22), 95(68), 93(26), 83(8), 81(20), 79(10), 69(10), 67(19), 57(15) and 55(30).

2. Methyl threo-12,13-dihydroxyoctadec-cis-enoate

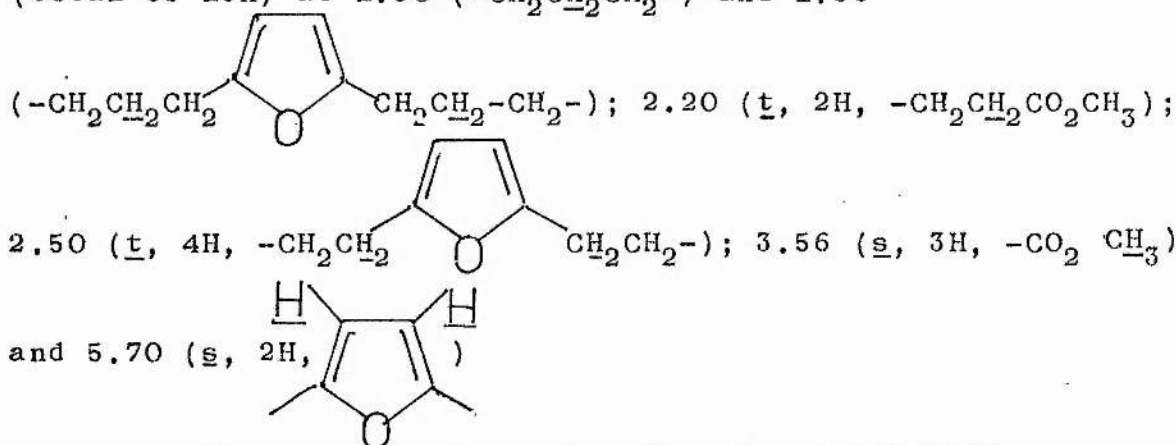
2.1 Reaction

Methyl threo-12,13-dihydroxyoctadec-cis-9-enoate (656 mg, 2.00 mmol), palladium (II) chloride (18 mg, 0.10 mmol) and cupric chloride (268 mg, 2.00 mmol) were reacted in dimethylformamide (15 ml) according to the usual procedure. After 20 min, water was added and the product (620 mg) was extracted with ether. GLC (DEGS) showed a single peak of ECL 21.1. TLC showed a major spot which had the same R_f value as an authentic C_{18} furanoid ester (10,13-furan). Prep. TLC (PE30) of a portion of the crude product (350 mg) afforded the pure material (223 mg, 64%).

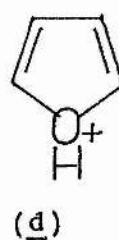
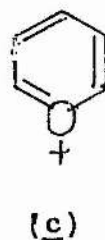
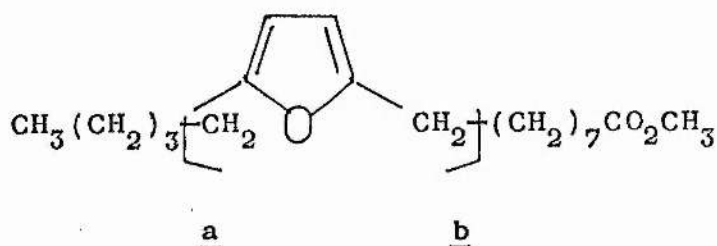
A portion of the product (450 mg) was separated by Prep. TLC (PE20) giving fractions A (180 mg, 52%), B (40 mg, 12%), and C (125 mg, 36%).

Fraction A

This compound of ECL 21.2 had a R_f value (TLC) similar to that of an authentic long-chain furanoid ester. Its UV spectrum showed an absorption maximum at 222 nm ($E_{\max} = 9,500$; hexane). The IR spectrum had absorption peaks at 3100 (f,w), 1610 (f,w), 1560 (f,s), 1010 (f,s), 950 (f,m), and 775 cm^{-1} (f,s), in addition to the signals usually associated with fatty esters. The PMR spectrum contained signals at 0.88 (t, 3H, CH_3CH_2-); multiplets (total of 16H) at 1.30 ($-\text{CH}_2\text{CH}_2\text{CH}_2-$) and 1.58

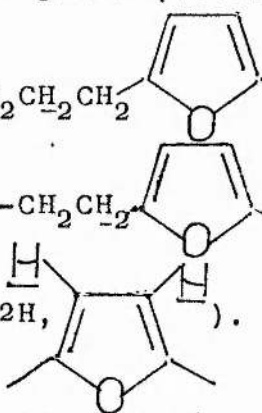


Its mass spectrum showed peaks at 308(M, 8), 277(M-31, 3), 251(a, 7), 165(b+14, 13), 151(b, 100), 95(a+b, 64), 81(c, 23), 74(31), and 69(d, 28).

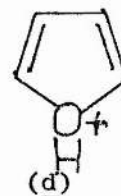
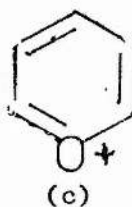
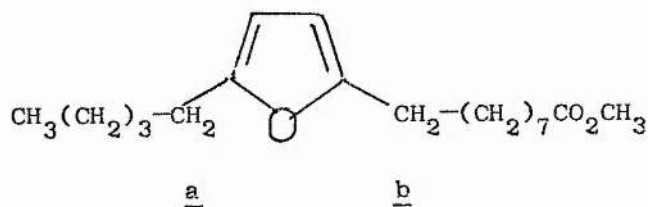


It showed a UV absorption maximum at 222 nm and its IR spectrum had absorption at 3100 (f,w), 1610 (f,w), 1560 (f,s), 1010 (f,s), 950 (f,m), and 775 cm^{-1} (f,s), in addition to the absorptions usually associated with fatty acid methyl esters. The PMR spectrum displayed signals at 0.90 (t, 3H, CH_3CH_2-); multiplets (total of 16H) at 1.32 ($-\text{CH}_2\text{CH}_2\text{CH}_2-$)

and 1.60 ($-\text{CH}_2\text{CH}_2\text{CH}_2-$); 2.22 (t, $-\text{CH}_2\text{CH}_2\text{CO}_2\text{CH}_3$); 2.52 (t, 4H, $-\text{CH}_2\text{CH}_2-$); 3.58 (s, 3H, $-\text{CO}_2\text{CH}_3$) and 5.72 (s, 2H, H).



Its mass spectrum displayed peaks at 308(M,7), 277(M-31,3), 251(a,7), 165(b+14,9), 151(b,100), 95(a+b,40), 81(c,23), 74(100), and 69(d,46).



It also had peaks associated with the ion fragments $-(\text{CH}_2)_n\text{CO}_2\text{CH}_3$ [87(30)], $\text{C}_n\text{H}_{2n+1}$ [57(100), 71(46), 85(21)], $\text{C}_n\text{H}_{2n-1}$ [55(100), 83(23), 97(15)], $\text{C}_n\text{H}_{2n-3}$ [67(23)] and the following additional peaks: 119(12), 105(21), 91(30).

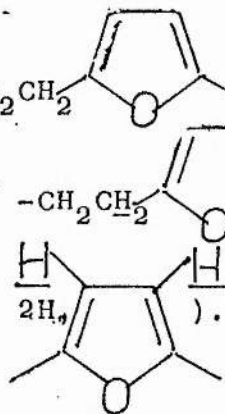
2.2 Silylation

After silylation, the products of the previous reaction showed mainly two peaks of ECL 21.0 (70%) and 21.6 (19%). The starting dihydroxy ester (as bis-OTMS ether) had an ECL of 21.6.

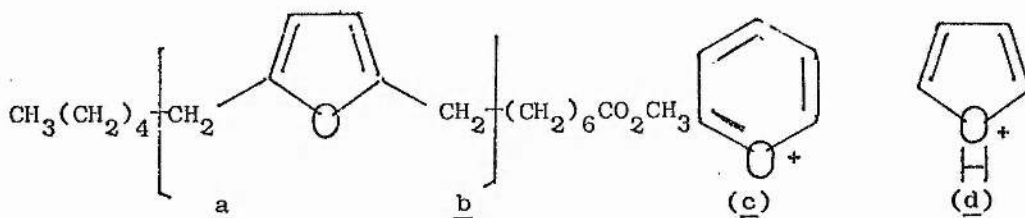
3. Methyl trans-11,12-epoxyoctadec-9-ynoate

A mixture of methyl trans-11,12-epoxyoctadec-9-ynoate (308 mg, 1.00 mmol), palladium (II) chloride (9 mg, 0.10 mmol) and anhydrous cupric chloride (135 mg, 1.00 mmol) in dimethylformamide solution were allowed to stand at room temperature, overnight. (Black palladium powder was deposited within seconds of mixing the reactants, indicating a fast reaction and therefore the reaction mixture was not heated as done normally). Water was then added and the product isolated in the usual way. GLC (SP 2300) showed a single peak of ECL 20.6. On TLC the product appeared as a single spot with the same R_f value as an authentic C_{18} furanoid methyl ester. Prep. TLC (PE30) afforded the pure product (175 mg, 57%), whose IR spectrum contained peaks at 3100 (f,w), 1610 (f,w), 1560 (f,s), 1010 (f,s), 950 (f,m), and 775 cm^{-1} (f,s), in addition to the absorption peaks usually observed in the spectra of saturated fatty acid methyl esters. Its UV spectrum had an absorption maximum at 222 nm and the PMR spectrum contained signals at 0.88 (t, 3H, CH_3CH_2-); multiplets (total of 16H) at 1.32 ($-\text{CH}_2\text{CH}_2\text{CH}_2-$) and

1.58 ($-\text{CH}_2\text{CH}_2\text{CH}_2-$); 2.22 (t, 2H, $-\text{CH}_2\text{CH}_2\text{CO}_2\text{CH}_3$); 2.52 (t, 4H, $-\text{CH}_2\text{CH}_2-$); 3.58 (s, 3H, $-\text{CO}_2\text{CH}_3$); and 5.72 (s, 2H, $-\text{CH}=\text{CH}-$).



Its mass spectrum displayed peaks at 308(M,10), 277(M-31,4), 251(a+14,2), 237(a,10), 179(b+14,9), 165(b,100), 95(a+b,70), 81(c,23), 74(10), and 69(d,15).



It also contained peaks associated with the ion fragments $\text{C}_n\text{H}_{2n+1}$ [57(25), 71(15), 85(9)], $\text{C}_n\text{H}_{2n-1}$ [55(35), 83(13), 97(6)], $\text{C}_n\text{H}_{2n-3}$ [67(12), 109(8), 123(5)], and the following additional peaks: 163(7), 121(8), 107(19), 94(16).

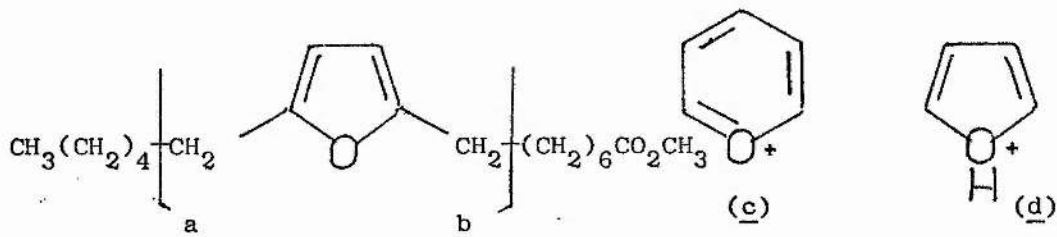
4. Methyl cis-9,10-epoxyoctadec-12-ynoate (616 mg, 2.00 mmol), palladium (II) chloride (36 mg, 0.20 mmol) and anhydrous cupric chloride (268 mg, 2.00 mmol) were reacted in dimethylformamide solution (15 ml), according to the usual procedure. The reaction was stopped after 30 min, and the product (573 mg) isolated in the usual way. GIC (SP 2300) of the product, however, did not show any peaks and TLC showed only polar material. Similar results were obtained when the reaction was carried out at room temperature overnight and the reaction was not investigated further.

5. Methyl 12-hydroxyoctadec-9-ynoate

Methyl 12-hydroxyoctadec-9-ynoate (122 mg, 0.39 mmol), palladium (II) chloride (7 mg, 0.04 mmol) and cupric chloride (53 mg, 0.39 mmol) were reacted for 20 min, in dimethylformamide (5 ml) according to the usual procedure. GLC (DEGS) examination of the product (a dark brown oil, 102 mg) showed two overlapping peaks of ECL 20.7 (49%) and 21.1 (30%) and other peaks of ECL 17.7 (11%) and 23.3 (10%). The product was separated by Prep. TLC (PE30) into fractions A (37 mg, 61%) and B (24 mg, 39%).

Fraction A

GLC of this fraction showed a single peak of ECL 20.7 and on TLC it gave a single spot whose R_f value was the same as that of an authentic C_{18} furanoid methyl ester. Its UV spectrum had an absorption maximum at 222 nm and the IR spectrum contained peaks at 3100 (f,w), 1610 (f,w), 1560 (f,s), 1010 (f,s) 950 (f,m), and 775 cm^{-1} (f,s), in addition to the usual fatty acid methyl ester absorptions. Its mass spectrum contained peaks at 308(M,6), 277(M-31,3), 251(a+14,1), 237(a,8), 179(b+14,7), 165(b,100), 95(a+b,72), 81(c,22), 74(10), and 69(d,21).

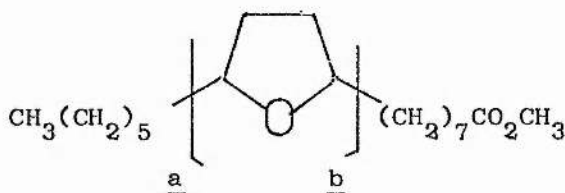


It also contained peaks associated with ion fragments $-(CH_2)_nCO_2CH_3$ [87(4)], C_nH_{2n+1} [57(72), 71(30), 85(18), 99(3), 113(5)], C_nH_{2n-1} [55(40), 83(12), 97(9), 111(4)], C_nH_{2n-3} [67(14), 109(8), 123(5)], and the following additional peaks: 123(5), 121(7), 119(5), 107(19), 79(9), 59(33).

Fraction B

GLC of fraction B showed peaks of ECL 17.7 (12%), 20.9 (67%) and 23.3 (21%). Its IR spectrum had a peak at 3460 (b,m) and strong absorption in the region 1710-1750 cm^{-1} . Silylation did not produce any new GLC peaks though the peak of ECL 20.9 disappeared almost completely.

Hydrogenation of fraction B gave a major product of ECL 20.4 (double peak). The hydrogenated product had GLC and TIC behaviour identical with that of an authentic sample of methyl 9,12-epoxyoctadecanoate. Its mass spectrum displayed peaks at 227(a,12), 209(a-18,7), 195(a-32,12), 177(a-50,7), 155(b,42), 137(b-18,25), and 74(62).



It also contained peaks associated with ion fragments $-(CH_2)_nCO_2CH_3$ [87(31)], C_nH_{2n+1} [57(68), 71(39), 85(33)], C_nH_{2n-1} [55(100), 69(71), 83(31), 97(32), 111(14)],

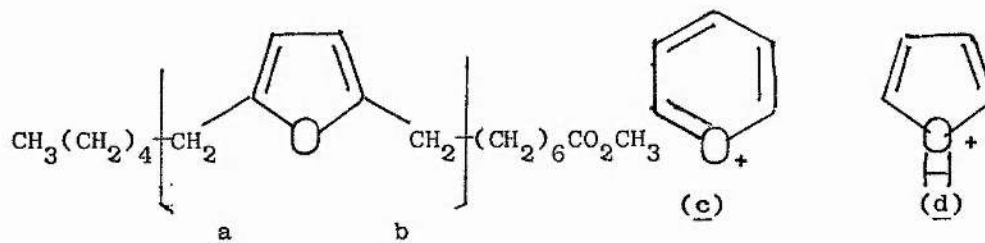
$C_n H_{2n-3}$ [67(52), 81(63), 95(70), 109(18)].

6. Methyl 12-hydroxyoctadec-cis-9-enoate

Methyl 12-hydroxyoctadec-cis-enoate (624 mg, 2.00 mmol), palladium (II) chloride (36 mg, 0.20 mmol) and cupric chloride (268 mg, 2.00 mmol) were reacted in dimethylformamide (15 ml) for 10 hrs, according to the usual procedure. Examination of the product (a dark brown oil, 574 ml) by GLC (DEGS), showed two overlapping peaks of ECL 20.8 (19%) and 20.9 (53%) as the major components. Other peaks had ECL values of 17.9 (10%) 18.5 (2%), 23.6 (2%) 24.1 (11%) and 24.6 (3%). A portion of the product (180 mg) was separated by Prep. TLC (P830) to obtain fractions: A (19 mg, 16%), B (71 mg, 61%), C (11 mg, 9%), D (16 mg, 14%).

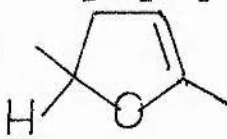
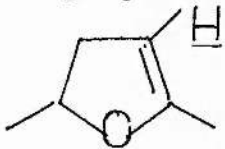
Fraction A

This compound had GLC and TLC properties identical with that of an authentic sample of 9,12-furan (ECL 20.8, R_f 0.67). Its IR spectrum showed peaks at 3100 (f,w), 1610 (f,w), 1560 (f,s), 1010 (f,s), 950 (f,m), and 775 cm^{-1} (f,s). The UV spectrum had an absorption maximum at 222 nm, and the mass spectrum displayed peaks at 308(M,2), 277(M-31,1), 237(a,2), 165(b,14), 95(a+b,17), 81(c,18), 74(42), 69(d,35)

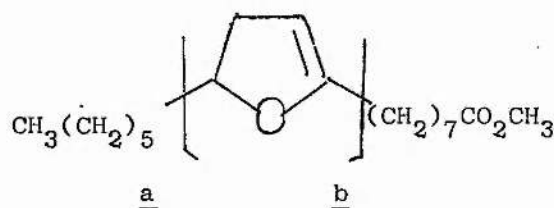


It also contained peaks associated with ion fragments $-(CH_2)_nCO_2CH_3$ [87(10)], C_nH_{2n+1} [57(100), 71(52), 85(22), 113(5)], C_nH_{2n-1} [55(80), 83(21), 97(14), 111(7), 125(4)], C_nH_{2n-3} [67(18), 109(7), 123(4), 137(3)], and the following additional peaks: 153(8), 119(8), 113(5), 105(10), 91(12), 79(6), 77(7) and 59(80).

Fraction B

GLC of fraction B showed peaks of ECL 20.8 (86%), 23.6 (2%) and 24.1 (12%), while its TLC (P330) showed a single spot (R_f 0.51). Though its IR spectrum did not display any unusual absorptions, the PMR spectrum contained signals at 0.88 (t, 3H, CH_3CH_2-); 1.30-1.5 (m, $-CH_2CH_2CH_2-$); 2.22 (t, 2H, $-CH_2CH_2CO_2CH_3$); 3.59 (s, 3H, $-CO_2CH_3$); ^{4.64}~~5.70~~ (s, 1H, ); 5.70 (s, 1H, ).

The mass spectrum of fraction B displayed peaks at 310(M,2), 279(M-31,2), 225(a,33), 193(a-32,1), 153(b,100), and 74(50).



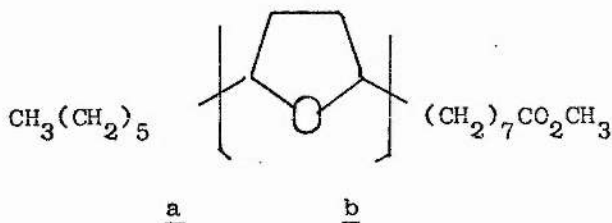
It also contained peaks associated with the ion fragments $-(CH_2)_nCO_2CH_3$ [87(11)], C_nH_{2n+1} [57(19), 71(7), 85(8)], C_nH_{2n-1} [55(60), 69(18), 83(15), 97(8)], C_nH_{2n-3}

[67(18), 81(35), 95(12), 109(5)], and the following additional peaks: 185(5), 165(6), 113(11), 93(5), 79(8) and 59(11).

Hydrogenation

A portion of fraction B (20 mg) was submitted to hydrogenation. Examination of the product by GLC showed a double peak of ECL 20.5 (70%) and 20.8 (23%) and other peaks of ECL 14.5 (2%), 16.0 (1%) and 18.0 (4%). Under the same GLC conditions, a sample of methyl 9,12-epoxyoctadecanoate prepared by the complete hydrogenation of authentic methyl 9,12-epoxyoctadec-9,12-dienoate showed a double peak of ECL 20.5 (76%) and 20.8 (24%).

Its mass spectrum displayed peaks at 227(a,3), 209(a-18,1), 195(a-32,3), 179(a-50,1), 155(t,8), 137(b-18,4) and 74(100).



It also contained peaks associated with the ion fragments $-(\text{CH}_2)_n\text{CO}_2\text{CH}_3$ [87(50), 101(5)], $\text{C}_n\text{H}_{2n+1}$ [57(56), 71(37), 85(14)], $\text{C}_n\text{H}_{2n-1}$ [55(85), 69(37), 83(19), 97(14)], $\text{C}_n\text{H}_{2n-3}$ [67(18), 81(20), 95(14)], and the following additional peaks: 165(7), 161(7), 125(5), 124(6), 59(18).

Fraction C

GLC (DEGS) of fraction C showed a peak of ECL 24.6, but the response was poor indicating that its major component(s) did not elute from the column under the conditions employed. TLC (PE30) showed a single spot of low R_f (0.18). Although its IR spectrum contained a absorption peak at 3450 cm^{-1} (b,m) and a rather broad peak in the carbonyl region, the material was unaffected by silylation or hydrogenation.

Fraction D

GLC (DEGS) showed a peak of ECL 18.3 but the response was again poor. On TLC (PE30), the material showed a single spot of low R_f value (0.14). Its IR spectrum contained absorption peaks at 3450 (b,m) and 1770 cm^{-1} (f,s). The GLC and TLC properties were unaltered when this fraction was submitted to silylation or hydrogenation.

7. Methyl 12-hydroxyoctadec-trans-9-enoate

Methyl 12-hydroxyoctadec-trans-9-enoate (312 mg, 1.00 mmol), palladium (II) chloride (9 mg, 0.05 mmol) and cupric chloride (134 mg, 1.00 mmol) were reacted in dimethylformamide (8 ml) according to the normal procedure. Although GLC indicated that all of the starting material had reacted after 20 min, it failed to show any significant product. The IR spectrum of the product contained an absorption peak at 3450 cm^{-1} (b,m) and broad absorption in the carbonyl region. TLC showed a series

of polar spots and this reaction product was not investigated further.

8. Methyl 12-oxooctadec-cis-9-enoate

Methyl 12-oxooctadec-cis-9-enoate (104 mg, 0.33 mmol), palladium (II) chloride (3 mg, 0.02 mmol) and anhydrous cupric chloride (47 mg, 0.33 mmol) were reacted in dimethylformamide (3 ml) according to the usual procedure. After 20 min, all of the oxo-ester had reacted but GLC (DEGS) did not show any significant product. TLC showed a series of polar spots and the reaction was not investigated further.

9. Methyl 9-hydroxyoctadec-cis-12-enoate

9.1 Reaction

Methyl 9-hydroxyoctadec-cis-12-enoate (624 mg, 2.00 mmol), palladium (II) chloride (18 mg, 0.10 mmol) and anhydrous cupric chloride (268 mg, 2.00 mmol) was reacted in dimethylformamide (15 ml) for 20 min. in the usual manner. The product (a dark brown oil, 620 mg) was examined by GLC (DEG) before and after silylation (Table 31). Attempts to isolate individual components are reported in section 9.4.

9.2 Catalytic Hydrogenation

A portion of the total reaction product (100 mg) was hydrogenated in the presence of palladized charcoal (10%, 5 mg). The reaction was carried out at room temperature and atmospheric pressure for 45 min, and the product

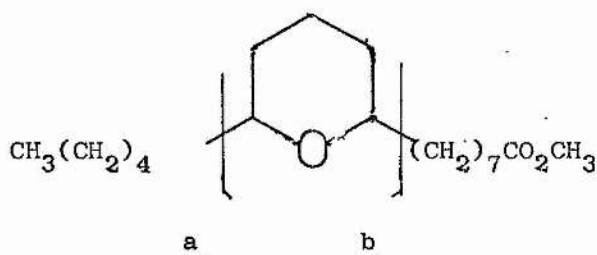
(92 mg) examined by GLC before and after silylation (Table 31) . Prep. TLC (P330) of the hydrogenated material (60 mg) afforded fractions A (5 mg, 10%), B (5 mg, 10%), C (26 mg, 51%) and D (15 mg, 29%).

Fraction A

This compound had GLC and TLC properties identical with that of methyl octadecanoate (ECL 18.0). Its mass spectrum displayed peaks at 298(M,15), 267(M-31,4), 255(M-43,7), 74(100) together with peaks associated with ion fragments $-(CH_2)_nCO_2CH_3$ [87(100), 101(9), 115(3), 129(8), 143(19), 157(2), 185(2), 199(4), 213(2), 241(1), 255(7)], C_nH_{2n+1} [57(67), 71(18), 85(8)], C_nH_{2n-1} [55(75), 69(4), 83(14), 71(11), 111(4)], and C_nH_{2n-3} [67(9), 81(7), 95(5), 109(2)].

Fraction B

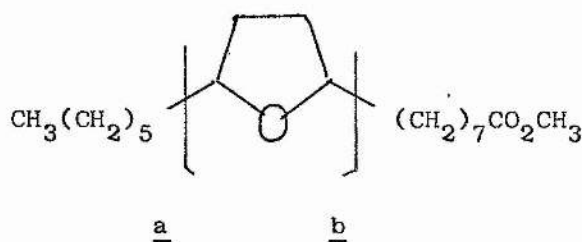
The mass spectrum of this compound (ECL 19.5) showed peaks at 312(M,0.5), 281(M-31,0.2), 269(M-43,0.2), 241(a,6), 223(a-18,7), 209(a-32,10), 191(a-50,5), 155(b,55) and 137(b-18,40), and 74(80).



It also displayed peaks associated with ion fragments $-(CH_2)_nCO_2CH_3$ [87(35)], C_nH_{2n+1} [57(55), 71(35), 99(6)], C_nH_{2n-1} [55(100), 69(95), 83(50), 97(11), 111(9), 125(4)], C_nH_{2n-3} [67(80), 81(32), 95(40), 109(18), 123(5)], and the following additional peaks: 173(7), 59(25), 54(37).

Fraction C

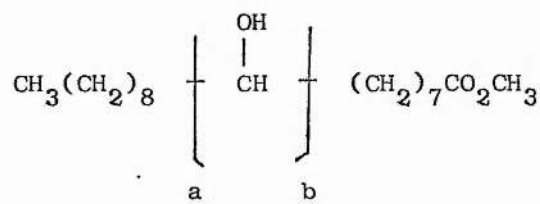
GLC of this compound showed a double peak of ECL 20.7 and 20.9. Apart from two weak signals in the fingerprint region (1090 and 880 cm^{-1}), its IR spectrum did not contain any unusual absorption peaks. Its mass spectrum displayed peaks at 321(M, 0.2), 281(M-31, 0.2), 227(a, 27), 209(a-18, 6), 195(a-32, 37), 177(a-50, 10), 155(b, 32), 137(b-18, 32), and 74(21).



It also contained peaks associated with fragment ions $-(CH_2)_nCO_2CH_3$ [87(12)], C_nH_{2n+1} [57(100), 71(14), 85(12), 99(5), 113(4)], C_nH_{2n-1} [55(100), 69(70), 83(32), 97(16), 111(8), 125(3)], C_nH_{2n-3} [67(75), 81(90), 95(77), 109(9)], and the following additional peaks: 200(5), 159(7), 149(9), 93(12).

Fraction D

GLC of this fraction showed a major peak of ECL 24.6. The mass spectrum contained peaks at 187(a,17), 158(b+1,23), 155(a-32,100), and 74(65).



It also contained peaks associated with ion fragments $-(CH_2)_nCO_2CH_3$ [87(78)], C_nH_{2n+1} [57(56), 71(21), 85(12)], C_nH_{2n-1} [55(96), 69(53), 83(46), 97(16), 111(7)], C_nH_{2n-3} [67(60), 81(23), 95(15), 109(18)], and the following additional peaks: 227(7), 195(12), 135(6), 129(7), 115(17).

9.3 Reduction with sodium borohydride

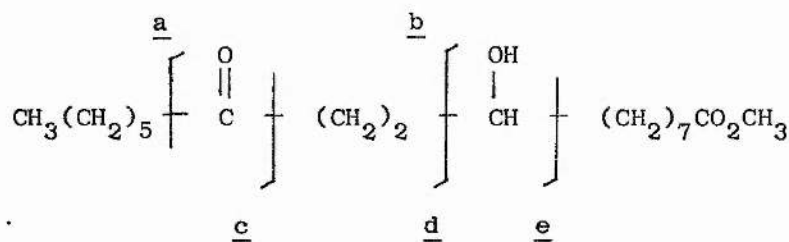
A portion of the reaction product (Section 9.1, 25 mg) was reduced with sodium borohydride and the product examined by GLC before and after silylation. Part of the reduction product (10 mg) was submitted to catalytic hydrogenation and the product similarly examined (Table 31).

9.4 Attempts to isolate individual components from
the reaction product

(a) Thin layer chromatography

The total reaction product (Section 9.1, 100 mg) was separated by Prep. TLC (PE30) into fractions: A (6 mg, 8%), B (4 mg, 5%), C (16 mg, 21%), D (42 mg, 57%) and

E (7 mg, 9%). On the basis of GLC, none of these fractions contained single compounds. Each contained a peak of ECL 21.4 which was the major component of fraction B, C and D. Only the largest fraction (D), was examined further. Rechromatography (PS20) of D afforded subfractions D₁ (8 mg, 23%) and D₂ (27 mg, 77%). GLC of subfraction D₂ showed two peaks of ECL 20.5 (10%) and 21.4 (90%). TLC showed a single spot (R_F 0.08) and appeared just behind the original hydroxy ester (R_F 0.10). The IR spectrum of subfraction D₂ showed absorption peaks at 3340 (b,m), 1710 (f,s), 930 (f,w), 980 (f,w), and 750 cm⁻¹ (f,w), in addition to the usual fatty acid methyl ester absorptions. Its mass spectrum contained peaks at 310(M-18, 5), 243(a,9), 211(a-32,15), 171(e,5), 156(b-31,15), 141(d,6), 113(c,29), and 74(15).



It also contained peaks associated with the fragment ions $-(\text{CH}_2)_n\text{CO}_2\text{CH}_3$ [87(8)], $\text{C}_n\text{H}_{2n+1}$ [57(32), 71(15), 85(20), 99(5)], $\text{C}_n\text{H}_{2n-1}$ [55(100), 69(30), 83(24), 97(15), 111(12), 125(5), 139(21), 153(5)], $\text{C}_n\text{H}_{2n-3}$ [67(33), 81(27), 95(18), 109(16), 128(8)], and the following additional peaks: 279(3), 267(3), 151(12), 135(10), 133(10), 98(16), 79(11), 68(12), 67(33), 59(11), 58(10).

(b)1 Crystallization

A portion of the reaction product (Section 9.1, 100 mg) was crystallized from petrol (1 ml) at -20° , overnight. Both GLC and TLC examination of the precipitate (46 mg) showed a single component (ECL 21.3, R_f 0.08). The IR spectrum contained absorption peaks at 3340 (b,m), and 1710 cm^{-1} (f,s) and its mass spectrum was similar to that of the subfraction D_2 [Section 9.4 (a)]. The PMR spectrum contained signals at 0.84 (t, CH_3CH_2) 1.26-1.6 (m, $-\text{CH}_2\text{CH}_2\text{CH}_2-$); 2.17 (t, $-\text{CH}_2\text{CH}_2\text{CO}_2\text{CH}_3$); 2.5 (broad signal, ?) and 3.54 (s, $-\text{CO}_2\text{CH}_3$).

(b)2 Silylation

The precipitate was silylated in the presence of an internal standard (methyl stearate) and examined by GLC before and after the reaction. The following relative areas of the GLC peaks are expressed with respect to methyl stearate (100).

	18:0	ECL 21.3	ECL 24.6	Other
Before silylation	100	100	-	trace
After silylation	100	13	218	12

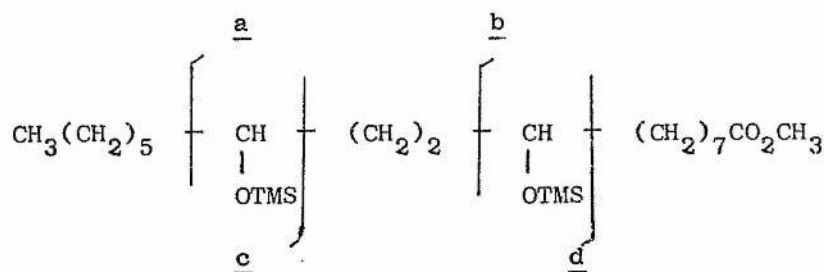
(b)3 Catalytic Hydrogenation

When submitted to catalytic hydrogenation, the precipitate [Section 9.4 (b)1, 10 mg] gave a product (8 mg) with GLC and TLC characteristics identical with that of authentic methyl 9,12-epoxyoctadecanoate (double peak of ECL 20.7 and 20.9, R_f 0.55). The mass spectrum of the product was also similar to that of the tetrahydrofuran-ester

(Section 9.2, Fraction C).

(b)4 Reduction with sodium borohydride

A portion of the precipitate Section (b)1, 20 mg was reduced with sodium borohydride but the product (18 mg) did not show any peaks on GLC (DEGS). Prep. TLC (PE60) of the reduction product afforded fraction A (1 mg, 7%), B (2 mg, 14%) and C (11 mg, 79%). Fractions A and B did not show GLC peaks either before or after silylation and were not examined further. After silylation, fraction C showed GLC peaks of ECL 22.0 (25%) and 22.4 (75%). The mass spectrum of the bis-OTMS ether contained peaks at 358(a-31,0.2), 317(d,2), 227(b-32,6), 187(c,12), 75(HO^+SiMe_2 ,100), 74(12) and 73(SiMe_2^+ ,100).



It also contained peaks associated with the ion fragments $\text{C}_n\text{H}_{2n+1}$ [57(100), 71(7), 85(5)], $\text{C}_n\text{H}_{2n-1}$ [55(55), 69(20), 83(12), 97(9), 111(3)], $\text{C}_n\text{H}_{2n-3}$ [67(17), 81(17), 109(7)], and the following additional peaks: 195(10), 155(7), 149(9), 147(12), 139(85), 95(13), 77(10), 76(14), 61(9), 59(9), 56(47).

9.5 Attempt to prepare methyl 12-hydroxy-9(10)-oxooctadecanoate

A solution of methyl 12-hydroxyoctadec-9-ynoate (100 mg, 0.32 mmol) and mercuric acetate (203 mg, 0.64 mmol) in methanol (20 ml) were allowed to stand in the dark at room temperature for 20 hrs. Methanol was removed under reduced pressure, replaced by chloroform and the solution filtered. After removal of the solvent for the second time, the residue was acidified with dilute hydrochloric acid (0.5 M, 10 ml) and extracted with ether to yield a product (100 mg) whose GLC showed a single peak of ECL 21.1, although TLC showed a series of spots. Prep. TLC (PE30) of the reaction product afforded fractions: A (10 mg, 15%), B (16 mg, 24%), and C (40 mg, 61%). Only fraction C was examined further.

Fraction C

GLC of fraction C showed a single peak of ECL 21.1 while TLC showed a polar spot (R_f 0.19). Its IR spectrum contained signals at 3450 (b,m) and 1710 cm^{-1} (f,s) in addition to the usual absorption peaks. The more prominent peaks of its mass spectrum were at: 310(3), 202(100), 201(44), 200(73), 199(55), 198(31), 181(42), 128(34), 127(18), 119(13), 111(18), 110(18), 101(13), 95(23), 83(36), 81(31), 69(55), 57(31) and 55(100).

III SYNTHESIS FROM 1,2-EPOXIDES

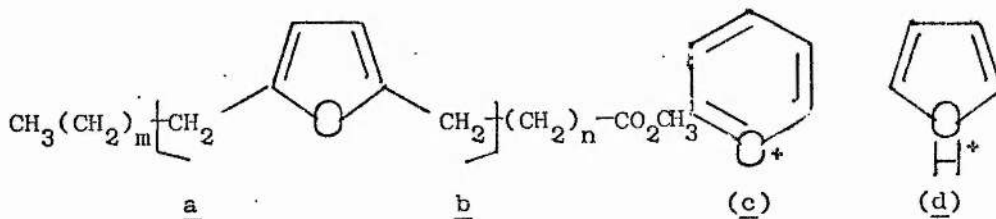
1. Methyl 9,10;12,13-diepoxyoctadecanoate

A mixture of methyl 9,10;12,13-diepoxyoctadecanoate (326 mg, 1.00 mmol), n-propyliodide (850 mg, 5.00 mmol), and sodium iodide (750 mg, 5.00 mmol) in dimethylsulphoxide (15 ml) was heated on a steam bath (90°). After 90 min. the reaction mixture was cooled, diluted with water, and the organic product (316 mg) was extracted with ether.

Examination of the product by GLC (SP 2300, 250°) showed peaks of ECL 20.8 (54%), 30.1 (38%), and 30.9 (8%). The product was separated into fractions A (97 mg, 42%), B (41 mg, 17%) and C (94 mg, 41%) by Prep. TLC (PE30). Only A and C were examined.

Fraction A

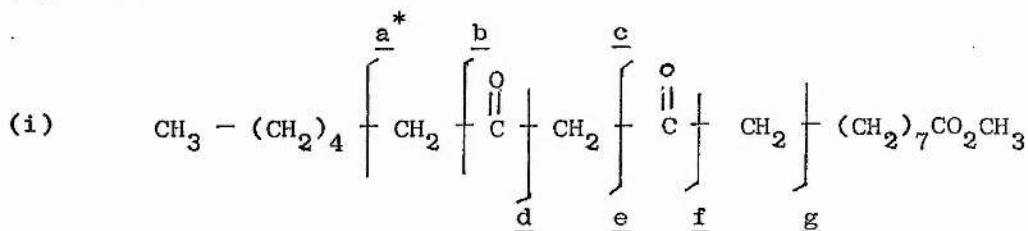
GLC of this compound showed a single peak of ECL 20.8 and its TLC behaviour was identical with that of an authentic C₁₈ furanoid methyl ester. Its UV spectrum had an absorption maximum at 222 nm and the IR spectrum showed peaks at 3100 (f,w), 1610 (f,w), 1560 (f,s), 1010 (f,s), 950 (f,m), and 775 cm⁻¹ (f,s) in addition to the usual fatty acid methyl ester signals. Its mass spectrum contained peaks at 308(M,11), 297(M-31,4), 251(a,n=7;6), 237(a,n=6;6), 165(b,n=6;61), 151(b,n=7:65), 95(a+b,100) 81(c,33), 74(7) and 69(d,21).



It also contained peaks associated with ion fragments, C_nH_{2n+1} [57(47), 71(26), 85(13), 99(7)], C_nH_{2n-1} [55(51), 83(13), 97(9)], C_nH_{2n-3} [67(18), 109(13)], and the following additional peaks: 121(10), 107(27), 94(24), 79(12).

Fraction C

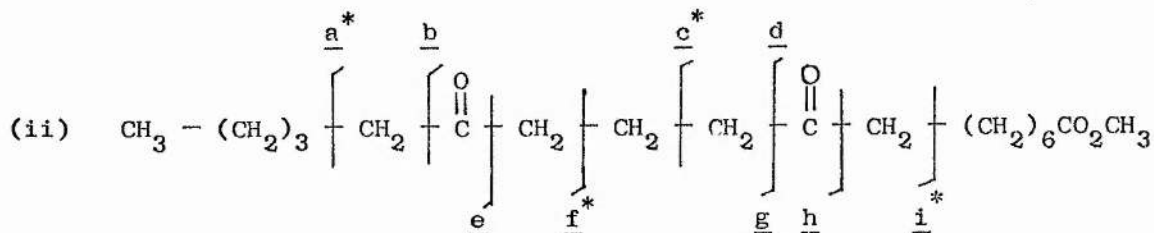
When examined by GLC (SP 2300, 250°) this fraction showed peaks of ECL 30.1 (85%) and 30.9 (15%), and its TLC (PE30) showed a single spot (R_f 0.33). Its IR spectrum had a peak at 1710 cm^{-1} (f,s) in addition to the ester carbonyl signal at 1740 cm^{-1} but absorptions due to hydroxyl functions were not observed. The PMR spectrum contained signals at 0.90 (t, 3H, CH_3CH_2-); multiplets (2OH) at 1.30 ($-CH_2CH_2CH_2-$) and 1.5 ($-CH_2CH_2CH_2CO-$); two partially overlapping triplets at 2.22 (2H, $-CH_2CH_2CO_2CH_3$) and (4H, $-CH_2CH_2CO-$); 2.54 (s, 2H, $-COCH_2CO-$) and 3.59 (s, 3H, $-CO_2CH_3$). Its mass spectrum displayed peaks at 326 (M, 1), 295 (M-31, 6), 256(a*, 2), 209(b-32, 6), 199(c, 5), 170(g*, 8), 155(f, 18), 127(e, 20) and 113(d, 16) associated with structure (i) and



* fragments arising from McLafferty rearrangement

• Ions common to both (i) and (ii)

at 326[•] (M,1), 295[•] (M-31,6), 270(a*,2), 223(b-32,10), 185(d,9), 184(i*,7), 169(c*-31 and h,10), 141(g,12), 114(f*,26) and 99(e,40) associated with structure (ii)



The mass spectrum also contained peaks associated with ion fragments - $(\text{CH}_2)_n \text{CO}_2 \text{CH}_3$ [87(10)]; $\text{C}_n \text{H}_{2n+1}$ [57(22), 71(50), 85(18)]; $\text{C}_n \text{H}_{2n-1}$ [55(100), 69(44), 83(25), 97(19), 111(15), 125(10)]; $\text{C}_n \text{H}_{2n-3}$ [67(22), 81(20), 95(18)]; 74(15) and the following additional peaks: 195(5), 59(18).

2. Diepoxides of methyl octadeca-cis-9,cis-12,cis-15-trienoate

A mixture of the three isomeric diepoxides of methyl octadeca-cis-9,cis-12,cis-15-trienoate (648 mg, 2.00 mmol) was heated at 90° with propyl iodide (1.70 g, 10.0 mmol) and sodium iodide (1.50 g, 10.0 mmol) in dimethylsulphoxide (30 ml). After 3 hrs, the reaction mixture was diluted with water and the organic product (630 mg) was extracted with ether. GLC (DEGS) examination of this material showed peaks of ECL 19.5 (3%), 20.7 (9%), 21.5 (6%), 21.7 (25%), 25.0 (3%), 26.2 (10%), 26.7 (19%) and 26.9 (25%). Several late eluting peaks (ECL > 30) were observed when the material was examined on a SP 2300 column

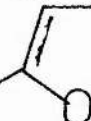
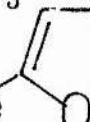
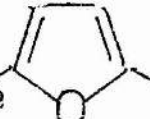
* fragments arising from McLafferty rearrangement

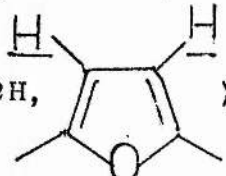
: Ions common to both (i) and (ii).

(250°) but the sum of them amounted to less than 10% of the total product. Prep. TLC (PE30) of a portion of the reaction product (300 mg) afforded fractions A (70 mg, 31%), B (81 mg, 36%) and C (73 mg, 33%).

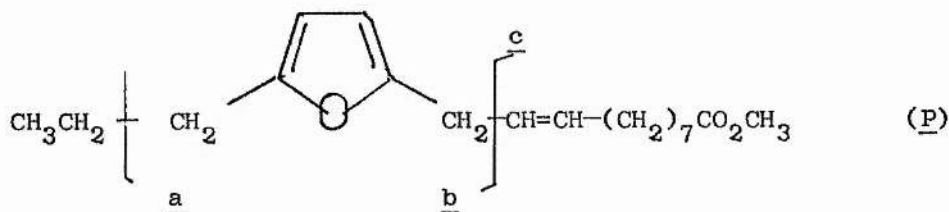
Fraction A

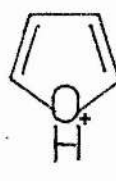
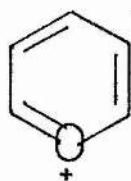
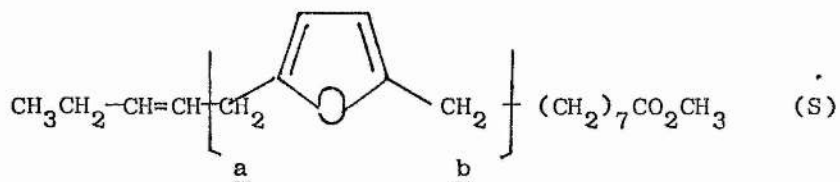
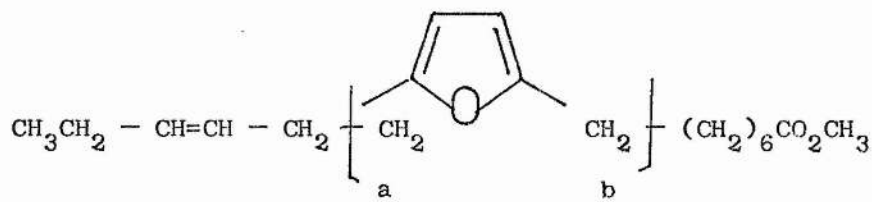
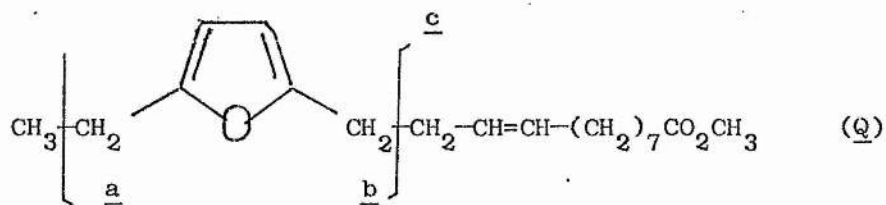
GLC (DEGS) of fraction A showed that it contained the components of ECL 19.5 (3%), 20.7 (17%), 21.5 (21%) and 21.7 (59%), and its TLC behaviour was similar to that of an authentic C₁₈ furanoid methyl ester. Its IR spectrum showed absorption peaks at 3100 (f,w), 3000 (f,w), 1610 (f,w), 1560 (f,s), 1010 (f,s), 950 (f,m), and 775 cm⁻¹ (f,s). The PMR spectrum contained overlapping triplets at 0.8-1.2 (3H) (CH₃CH₂CH₂- and CH₃CH₂CH=CH-); multiplets at 1.3 and 1.6 (~15H) (-CH₂CH₂CH₂-); two overlapping triplets at 2.2 (3H,

-CH₂CH₂CO₂CH₃ and -CH=CH-CH₂CH₂-); triplet at 2.5 (3H, -CH₂CH₂-); multiplet at 3.3 (1H, -CH=CH-CH₂-).

singlet at 3.58 (3H, -CO₂CH₃); multiplet at 5.3-5.5 (2H, -CH₂CH=CHCH₂-) and singlet at 5.74 (2H, ). The

UV spectrum of fraction A had an absorption maximum at 222 nm. Its mass spectrum displayed the following peaks





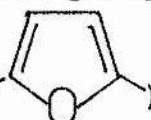
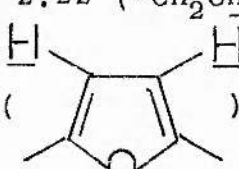
306(M,5), 275(M-31,2), 95(a+b,50), 81(d,22), 69 (e,11)

	<u>P</u>	<u>Q</u>	<u>P</u>	<u>S</u>
a	277(2)	-	237(7)	-
b	123(7)	109(100)	163(7)	149(17)
c-32	151(9)	165(8)	-	-

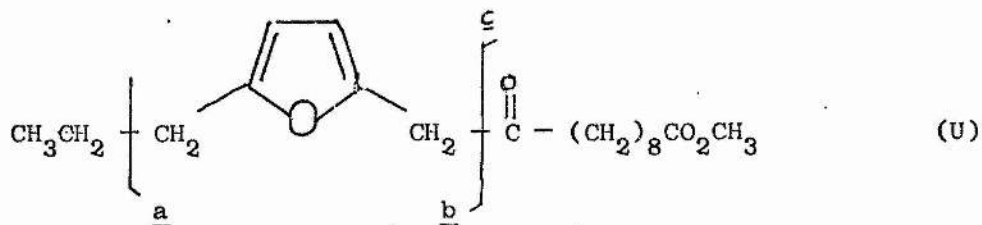
Others: 94(12), 91(9), 79(10), 77(7), 74(5), 71(5), 67(10), 59(8), 57(6) and 53(8).

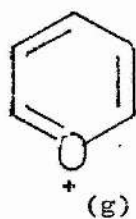
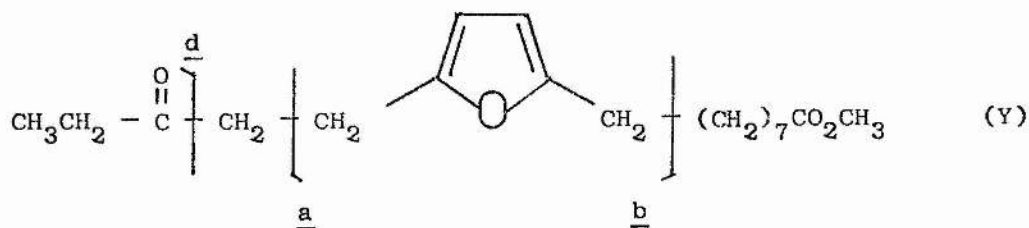
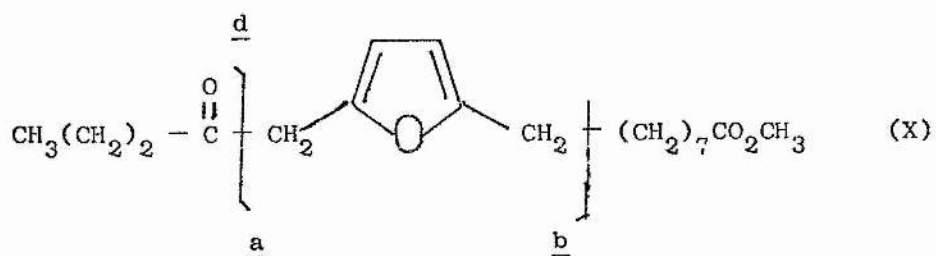
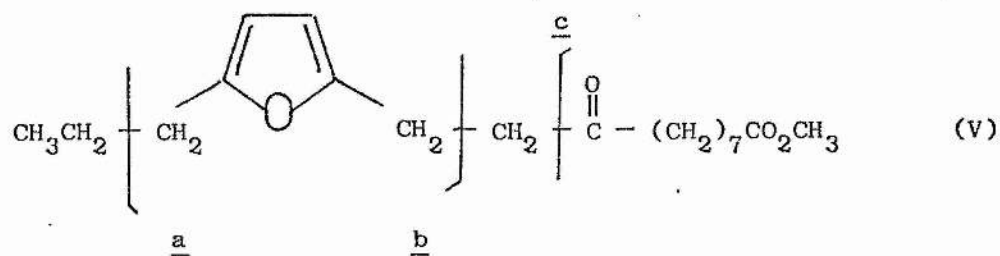
Fraction B

Although TLC of this fraction showed a single spot which had a R_f value similar to that of methyl 12-oxooleate, its GLC (DEGS) showed three incompletely resolved peaks of ECL 26.2 (18%), 26.7 (39%) and 26.9 (43%). When chromatographed on a SP 2300 column at 250°, the same peaks had ECL's of 26.5, 27.0 and 27.2. The IR spectrum of B contained peaks at 3440 (b,w), 3100 (f,w), 1720 (f,s), 1610 (f,w), 1560 (f,s), 1010 (f,s), 950 (f,m) and 775 cm^{-1} (f,s), in addition to the absorptions usually associated with fatty acid methyl esters. Its PMR spectrum contained two overlapping triplets at 0.86-1.10 ($\text{CH}_3\text{CH}_2\text{CH}_2-$ and $\text{CH}_3\text{CH}_2\text{CO}-$); multiplets at 1.3 and 1.6 ($-\text{CH}_2\text{CH}_2\text{CH}_2-$); a complex group of signals at 2.16-2.90 ($-\text{CH}_2\text{CO}$ and

$-\text{CH}_2$ ); a triplet at 2.22 ($-\text{CH}_2\text{CH}_2\text{CO}_2\text{CH}_3$) and singlets at 3.60 ($-\text{CO}_2\text{CH}_3$) and 5.76 ().

The mass spectrum of fraction B displayed the following peaks





322(M,9), 291(M-31,4), 95(a+b,38), 81(g,27), 74(18) and 69(h,19).

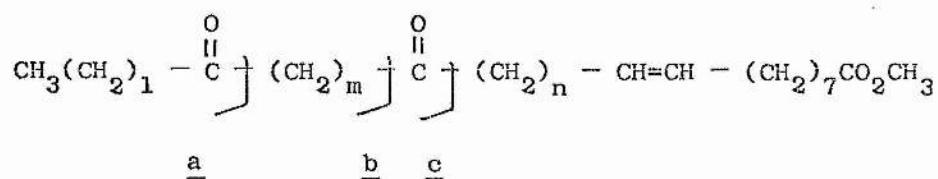
	(U)	(V)	(X)	(Y)
a	-	-	251(5)	251(5)
a-32	261(1)	261(1)	219(1)	219(1)
b	123(100)	123(100)	165(29)	165(29)
c	199(3)	185(4)	-	-
d	-	-	71(23)	57(45)

Also, there were peaks associated with ion fragments $-(CH_2)_nCO_2CH_3$ [87(11)], C_nH_{2n-1} [55(78), 83(15), 97(15), 111(6), 125(8)], C_nH_{2n-3} [67(19), 109(21)], and the following additional peaks: 149(6), 137(18), 107(32), 94(16), 77(9), 59(16).

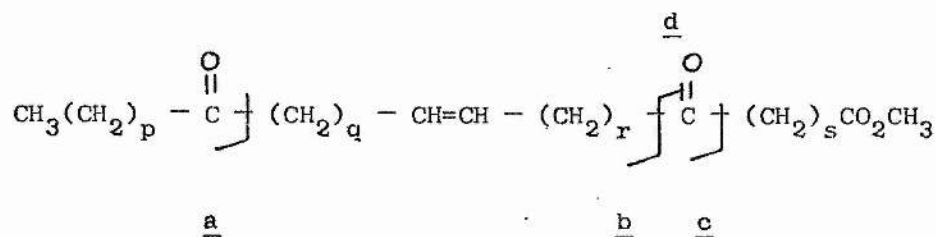
Fraction C

Silicic acid TLC of this material showed a spot which had a R_f value similar to that of methyl-9,12-dioxo-octadecanoate and its GLC (SP 2300) showed peaks of ECL 29.0 (12%), 29.6 (18%) and 31.0 (70%). Its IR spectrum showed absorption peaks at 3480 (b,m), 3000 (f,m), 1720 (f,s), 1680 (f,s), and 1580 cm^{-1} (f,s) in addition to the usual fatty acid methyl ester signals. The PMR spectrum contained signals at 0.80-1.14 (complex, 3H, $\underline{CH_3}CH_2-$), 1.30-1.8 (multiplets, 14H, $-CH_2CH_2CH_2-$), 2.14-2.56 (complex, 8H, $-CH_2CH_2CO_2CH_3$ and $-CH_2CO-$), 3.58 (s, 3H, $-CO_2\underline{CH_3}$) and 5.2-5.4 (complex, 2H, $-CH=CH-$).

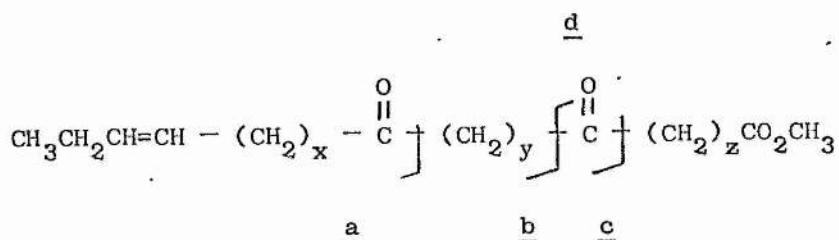
The mass spectrum of fraction C displayed the following peaks.



Position of oxo groups	a	b	c
12, 15	71(46)	99(7)	127(12)
13, 15	71(46)	85(8)	113(10)
12, 16	57(100)	99(7)	127(12)
13, 16	57(100)	85(8)	113(10)



Position of oxo groups	a	b	c	d
9, 15	71(46)	139(9)	-	185(13)
10, 15	71(6)	125(18)	-	199(3)
9, 16	57(100)	139(9)	-	185(13)
10, 16	57(100)	125(18)	-	199(3)



Position of oxo groups	a	b	c	d
9, 12	111(13)	139(9)	-	185(13)
10, 12	111(13)	125(18)	-	199(3)
9, 13	97(20)	139(9)	-	185(13)
10, 13	97(20)	125(18)	-	199(3)

The mass spectrum also contained peaks associated with ion fragments $\text{C}_n\text{H}_{2n-1}$ [55(81), 69(32), 83(21)], $\text{C}_n\text{H}_{2n-3}$ [67(37), 81(23), 95(22), 109(10), 123(15)], 74(14) and the following additional peaks: 251(2), 221(4), 121(6), 107(9), 93(8), 59(15).

3. Methyl 12,13-epoxyoctadec-cis-9-enoate

A mixture of methyl 12,13-epoxyoctadec-cis-9-enoate (620 mg, 2.00 mmol), propyl iodide (1.70 g, 10.00 mmol) and sodium iodide (1.50 g, 10.00 mmol) in dimethylsulphoxide (30 ml) was heated at 90°C for 3h. The reaction mixture was cooled overnight and washed with aqueous sodium thiosulphate (5%, 30 ml) to remove the iodine liberated during the reaction. Extraction with ether yielded a product (618 mg) whose GLC (SP 222) showed peaks of ECL 21.2 (44%), 24.7 (45%, broad), 26.1 (1%) and 28.5 (10%), which were unaffected by silylation. TLC showed a series of at least six spots, the least polar

of which had the same R_f value as that of authentic 9,12-furan. The reaction product (500 mg) was separated into fractions A (175 mg, 37%), B (180 mg, 38%), C (57 mg, 12%) and D (60 mg, 13%) by Prep. TLC (PE20). Fractions A and B were examined in detail.

Fraction A

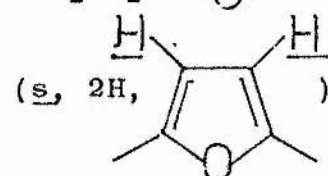
GLC and TLC behaviour of this compound was identical with that of authentic 9,12-furan (ECL 21.2). Its IR spectrum had absorption peaks at 3100 (f,w), 1610 (f,w) 1560 (f,s), 1010 (f,s), 950 (f,m) and 775 cm^{-1} (f,s) in addition to the usual signals. Its UV spectrum showed an absorption maximum at 222 nm while its PMR spectrum

contained signals at 0.88 (t, 3H, CH_3CH_2-); multiplets

(total 18H) at 1.32 ($-\text{CH}_2\text{CH}_2\text{CH}_2-$) and 1.58 ($-\text{CH}_2\text{CH}_2\text{CH}_2-$)

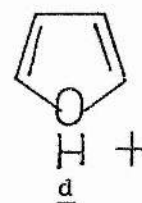
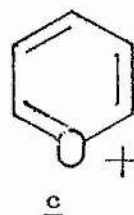
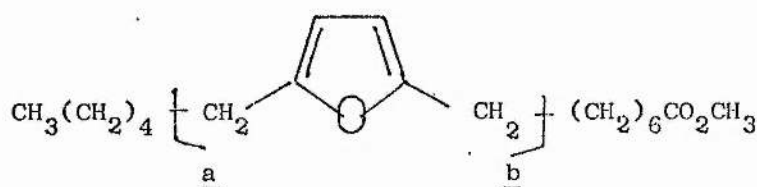
$\text{CH}_2\text{CH}_2\text{CH}_2-$); 2.20 (t, 2H, $-\text{CH}_2\text{CH}_2\text{CO}_2\text{CH}_3$); 2.52 (t, 4H,

$-\text{CH}_2\text{CH}_2-$); 3.58 (s, 3H, $-\text{CO}_2\text{CH}_3$) and 5.70



The mass spectrum of A displayed peaks at 308(M,11),

277(M-31,5), 237(a,13), 179(b+14,19), 165(b,100), 95(a+b,85), 81(c,45), 74(13) and 69(d,25).

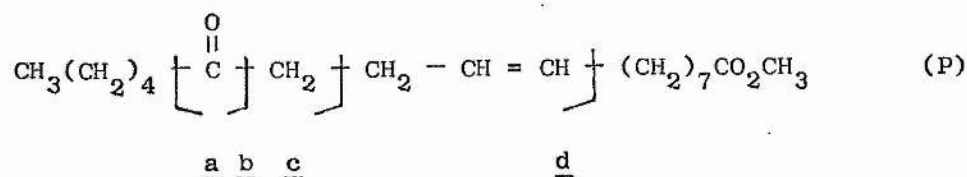


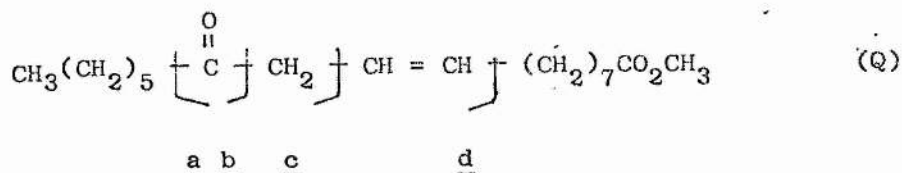
It also contained peaks associated with ion fragments C_nH_{2n+1} [57(14), 71(9)], C_nH_{2n-1} [55(74), 83(11)], C_nH_{2n-3} [67(28), 109(18), 123(7)], and additional peaks at 149(5), 135(8), 121(18), 113(10), 107(60) 91(14), 79(28), 67(28), 59(34) and 53(19). Peaks arising from the isomeric 10,13-furan were also present but were relatively small: 251(a,2) and 151(b,7).

Fraction B

GLC (SP 222) of this fraction showed a broad peak of ECL ~ 24.8, indicative of two overlapping components and under the same chromatographic conditions authentic methyl 12-oxooctadec-cis-9-enoate had an ECL of 24.75. The IR spectrum of B showed strong absorption at 1720 cm^{-1} , in addition to the usual ester carbonyl signal at 1740 cm^{-1} . Its PMR spectrum contained signals at

0.88 (t, 3H, CH_3CH_2-); 1.30-1.7 (m, 16H, $-\text{CH}_2\text{CH}_2\text{CH}_2-$); multiplets (10H) at 2.0 and 2.14-2.40 ($-\text{CH}=\text{CHCH}_2\text{CH}_2-$ and $-\text{COCH}_2\text{CH}_2-$ respectively); 3.03 (d, 1H, $-\text{COCH}_2\text{CH}=\text{CH}$); 3.58 (s, 3H, $-\text{CO}_2\text{CH}_3$); 5.26 (m), and 5.46 (t) (total 2H) ($-\text{COCH}_2\text{CH}_2\text{CH}=\text{CHCH}_2$ and $-\text{COCH}_2\text{CH}=\text{CHCH}_2-$ respectively). The mass spectrum of B displayed the following peaks





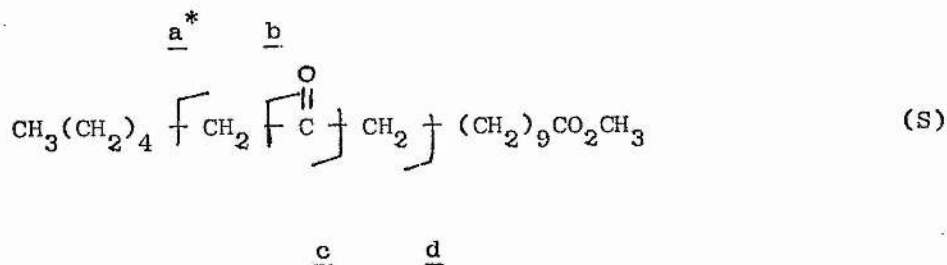
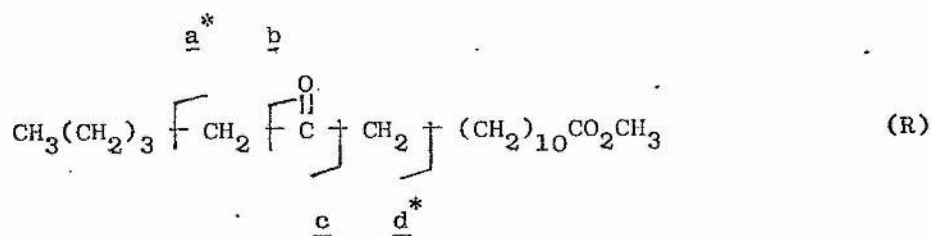
	<u>P</u>	<u>Q</u>
a	-	-
a-32	207(11)	-
b	99(100)	113(100)*
c	113(100)*	-

* Ions common to both P and Q

The mass spectrum also contained peaks associated with ion fragments $-(\text{CH}_2)_n\text{CO}_2\text{CH}_3$ [87(21)], $\text{C}_n\text{H}_{2n+1}$ [57(20), 71(41), 85(29)], $\text{C}_n\text{H}_{2n-1}$ [55(83), 69(37), 83(25), 97(14)], $\text{C}_n\text{H}_{2n-3}$ [67(50), 81(54), 95(37), 109(12), 123(12)], and the following additional peaks: 179(11), 165(20), 151(16), 136(10), 131(14), 121(10), 117(20), 107(10), 93(11), 91(11), 79(21), 61(18), 59(29).

Hydrogenation of Fraction B

When B was submitted to hydrogenation using palladized carbon as catalyst, a white solid was obtained whose GLC (SP 222) showed a single peak of ECL 24.4. The mass spectrum of the hydrogenation product displayed the following peaks.



	<u>R</u>	<u>S</u>
M-31	281(5)	281(5)
a	256(4)	-
b	241(8)	-
c	99(73)	113(24)
d	114(18)	128(11)

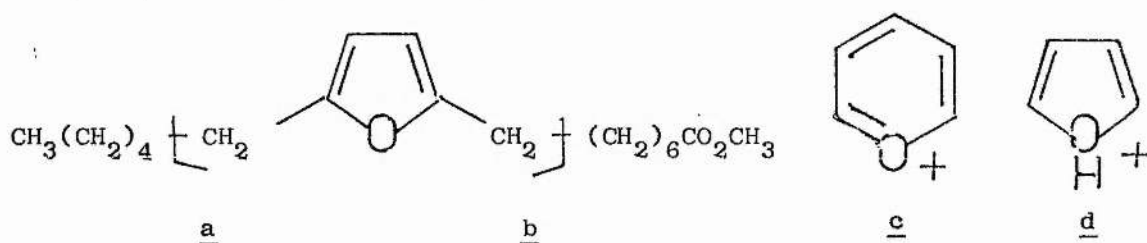
The mass spectrum also contained peaks associated with ion fragments $-(\text{CH}_2)_n \text{CO}_2 \text{CH}_3$ [87(38), $\text{C}_n \text{H}_{2n+1}$ [57(32), 71(100), 85(26), 127(11), 141(7)], $\text{C}_n \text{H}_{2n-1}$ [55(100), 69(80), 83(43), 97(36), 111(14), 125(9)], $\text{C}_n \text{H}_{2n-3}$ [67(38), 81(36), 95(24)], 74(66) and the following additional peaks: 199(9), 185(6), 167(9), 153(6), 149(7), 141(7), 126(9), 98(43), 58(60).

* These ions result from McLafferty rearrangement.

4. Methyl 12-oxooctadec-cis-9-enoate

Methyl 12-oxooctadec-cis-9-enoate (60 mg, 0.19 mmol), propyl iodide (164 mg, 0.96 mmol) and sodium iodide (145 mg, 0.96 mmol) were heated with dimethylsulphoxide (3 ml) at 90°C, for 3h. Usual work up yielded a product (57 mg) whose GLC (SP 222) showed a major peak of ECL 21.1. Purification of the product by Prep. TLC (PE20) furnished a material (11 mg, 19%) which had GLC and TLC properties identical with that of 9,12-furan (ECL 21.1, R_F 0.83). Its IR spectrum contained peaks at 3100 (f,w), 1610 (f,w), 1560 (f,m), 1010 (f,m), and 775 cm^{-1} (f,s) in addition to the usual absorptions.

Its mass spectrum displayed peaks at 308(M,4), 277(M-31,2), 237(a,3), 179(b+14,2), 165(b,52), 95(a+b,28), 81(c,13), 74(100) and 69(d,21)



The mass spectrum also contained peaks associated with fragment ions $\text{---}(\text{CH}_2)_n\text{CO}_2\text{CH}_3$ [87(58), 101(4)], $\text{C}_n\text{H}_{2n+1}$ [57(36), 71(11), 85(5), 99(3)], $\text{C}_n\text{H}_{2n-1}$ [55(58), 83(11), 97(7)], $\text{C}_n\text{H}_{2n-3}$ [67(12), 81(13), 109(4)], and the following additional peaks: 255(1), 199(1), 149(3), 121(4), 94(8).

5. Methyl 12-hydroxyoctadec-cis-9-enoate

Methyl 12-hydroxyoctadec-cis-9-enoate (314 mg, 1.0 mmol), n-propyl iodide (850 mg, 5.0 mmol) and sodium iodide (750 mg, 5.0 mmol) were heated with dimethylsulphoxide (15 ml) at 90°C. Aliquots were drawn at 2 hr intervals and examined by GLC and TLC. The results showed that no reaction had taken place even after a period of 6 hr.

IV SYNTHESIS VIA 1,4-ENDOPEROXIDES

1. Methyl octadeca-trans-8,trans-10-dienoate

(a) Sensitized photooxidation

Oxygen was bubbled through a methanolic solution of methyl octadeca-trans-8,trans-10-dienoate (139 mg, 0.47 mmol) and methylene blue for 5 days. During this time the solution was irradiated with two tungsten lamps (150 w each), and the reaction mixture was kept cool by means of a cold finger. Removal of solvent under reduced pressure gave a product (143 mg). Its GLC (SP 222) showed one broad peak only (ECL ~ 21.2), although TLC showed two well separated spots and also some polar material. Prep. TLC (PE20) afforded fractions A (17 mg, 14%) and B (86 mg, 74.) and C (14 mg, 12%). C consisted of polar material and only fractions A and B were examined.

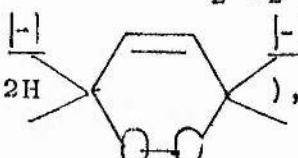
Fraction A

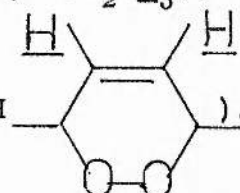
Whilst TLC of this fraction showed a single spot which had the same R_f as the original diene ester, its GLC (SP 222) showed two peaks of ECL 20.3 (20%) and 21.0 (80%). Its IR spectrum contained peaks at 3010 (f,s) and 990 cm^{-1} (f,s). When submitted to further reaction, fraction A produced more of material B, thus confirming that it contained unreacted starting material.

Fraction B

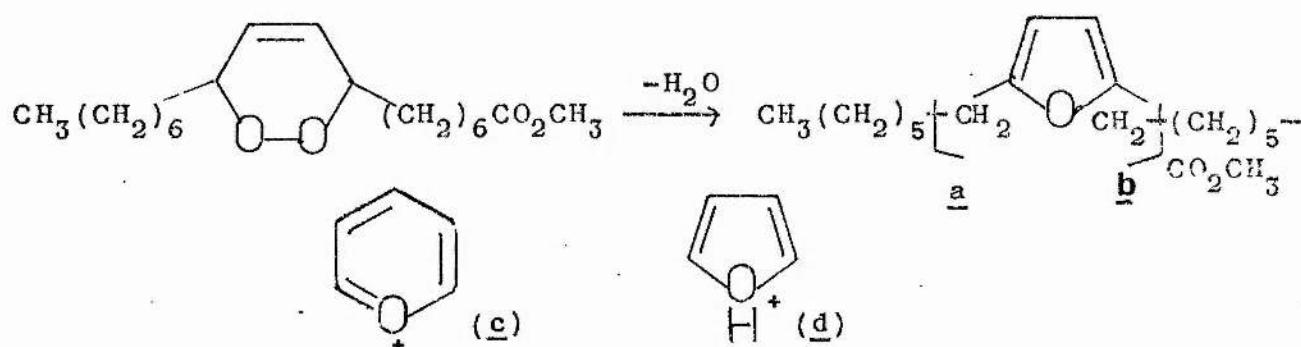
GLC (SP 222) of fraction B showed a peak of ECL 21.2. Its IR spectrum contained peaks at 3020 (f,m),

1695 (f,m), 880 (b,w), 790 (f,w), and 720 cm^{-1} (f,s). Its PMR spectrum contained signals at 0.88 (t, 3H, CH_3CH_2-); 1.30-1.7 (m, $-\text{CH}_2\text{CH}_2\text{CH}_2-$); 2.22 (t, 2H, $-\text{CH}_2\text{CH}_2\text{CO}_2\text{CH}_3$);

3.60 (t, 3H, $-\text{CO}_2\text{CH}_3$); 4.32 (m, 2H, ) and

5.80 (s, 2H, ). The mass spectrum of fraction B

displayed peaks at 308 (M-18,1), 294 (M-32,1), 223 (a,3), 179 (b,29), 95 (a+b,71), 81 (c,70), 69 (d,71). It also had minor peaks at 237 and 165 respectively, which are the corresponding a and b ion fragments arising from the isomeric 9,12-cycloperoxide.



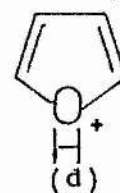
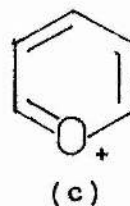
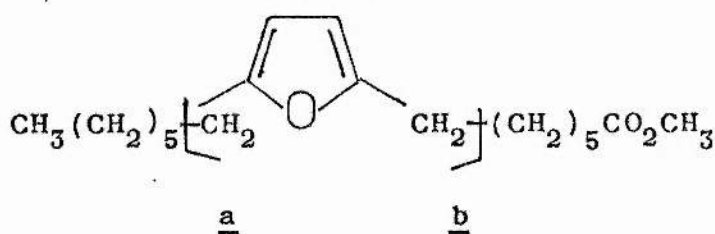
Presumably, small amounts of this isomer were produced from contamination of the original diene ester with methyl octadeca-trans-9,trans-11-dienoate. The mass spectrum had additional peaks at: 193(6), 149(8), 121(10), 111(10), 107(28), 83(43), 79(28), 74(28), 71(31), 67(57), 59(33), 57(100), and 55(100).

(b) Reduction of Fraction B

A solution of hydrated ferrugous sulphate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 127 mg, 0.46 mmol) in water (3.5 ml) was added, all at once, to a solution of fraction B (75 mg, 0.23 mmol) in THF (3 ml) and stirred at room temperature for 5 h. After removal of THF under reduced pressure, the reaction mixture was diluted with water. Acidification (to facilitate removal of the ferric sulphate formed) followed by extraction with ether yielded the organic product (62 mg) whose GLC (SP 222) showed a broad peak of ECL ~ 21.1 . TLC, however, showed two well separated spots and Prep. TLC (PE 20) of the material afforded fractions P (34 mg, 72%) and Q (13 mg, 28%).

Fraction P

TLC behaviour of fraction P was identical with that of authentic 9,12-furan and its GLC (SP 222) showed a single peak of ECL 21.1. Its UV spectrum had an absorption maximum at 222 nm and the IR spectrum contained peaks at 3100 (f,w), 1610 (f,w), 1560 (f,s), 1010 (f,s), 960 (f,m), and 775 cm^{-1} (f,s). Its mass spectrum displayed at 308 (M, 28), 277 (M-31, 10), 223 (a, 20), 179 (b, 100), 95 (a+b, 100), 81 (c, 40) and 69 (d, 19).



It also contained minor peaks at 237 (a) and 165 (b) arising from the isomeric 9,12-furan which might have been produced from contamination of the original diene ester with methyl octadeca-trans-9,trans-11-dienoate. The following additional peaks were also present: 199(10), 193(22), 149(20), 135(11), 121(19), 107(66).

Fraction Q

This fraction showed GLC and TLC behaviour identical with that of the original cycloperoxide (ECL 21.2). That fraction B was unconverted endoperoxide was confirmed by its IR and mass spectra.

CHEMICAL REACTIONS OF FURANOID FATTY ACIDS AND ESTERS

Unless stated otherwise all reactions reported in this section were performed on 10,13-furan acid or its methyl ester.

1. Acid-Catalysed Ring Opening

1.1 Hot methanolic sulphuric acid:

A mixture of the furanoid acid (270 mg, 0.91 mmol) and stearic acid (30 mg) was refluxed with methanolic sulphuric acid (2% w/v, 25 ml). After one hour, an aliquot was removed and prepared for GLC. The reaction was continued for a further one hour, whereupon water was added and the product (267 mg) extracted with ether. TLC (PE30) of the reaction product showed two spots, the less polar one of which had a R_f value similar to that of the original furanoid ester, and GLC (SP 2340, 230°) gave the following results.

ECL	Area (weight %)	
	<u>1h</u>	<u>2h</u>
18.0	8	8
22.4	51 (56)*	53 (57)
33.8	41 (44)	39 (43)

* The figure in parenthesis show the relative areas of the two products after correction has been made for the internal standard.

1.2(a) Methanolic sulphuric acid at room temperature

A methanolic solution (6 ml) of the furanoid acid (50 mg, 0.17 mmol) containing sulphuric acid (2%, w/v), was allowed to stand at room temperature, overnight. Usual work up afforded a product (47 mg) with the following GLC (SP 2340, 230°) results.

<u>ECL</u>	<u>Area (weight %)</u>
22.4	59
33.8	41

Prep. TLC (PE30) of the reaction product 1.1 gave fractions: A (157 mg, 65%) and B (83 mg, 35%).

Fraction A

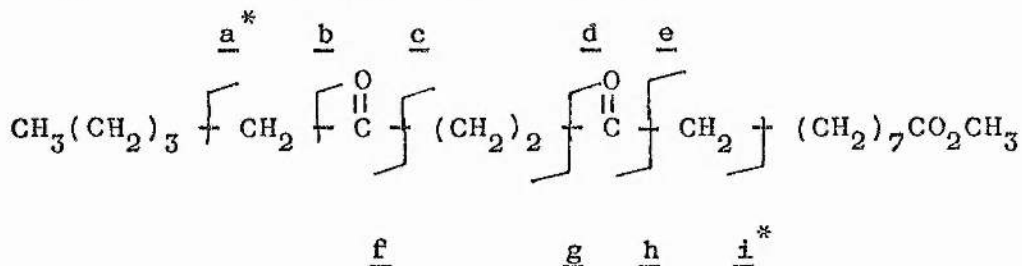
TLC (PE30) of fraction A showed a single spot whose R_f (0.83) was the same as that of the original furanoid ester and GLC showed peaks of ECL 18.0 (19%) and 22.4 (81%). Its IR spectrum had all the absorptions characteristic of C_{18} furanoid methyl esters and the UV spectrum contained an absorption maximum at 222 nm.

Fraction B

Both GLC (SP 2340, 230°) and TLC (PE30) of B indicated a pure compound (ECL 33.8, R_f 0.33) and its IR spectrum showed a peak at 1710 cm^{-1} (f,s) in addition to the normal fatty acid methyl ester signals. The PMR spectrum contained signals at 0.90 (s, 3H, CH_3CH_2-), 1.3 (m, 12H, $-\text{CH}_2\text{CH}_2\text{CH}_2-$), 1.5 (m, 6H, $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CO}-$), 2.14-2.44 (group of five signals, 6H, $-\text{CH}_2\text{CH}_2\text{CO}-$), 2.54

(s, 4H, $-\text{COCH}_2\text{CH}_2\text{CO}-$), and 3.59 (s, 3H, $-\text{CO}_2\text{CH}_3$).

The mass spectrum of B displayed peaks at 326 (M, 2), 295 (M-31, 10), 270 (\underline{a}^* , 7), 227 (\underline{c} , 6), 223 (\underline{b} -32, 33), 199 (\underline{d} , 19), 195 (\underline{c} -32, 16), 170 (\underline{i}^* , 29), 167 (\underline{d} -32, 13), 155 (\underline{h} , 38), 139 (\underline{e} -32, 18), 127 (\underline{g} , 39), 99 (\underline{f} , 55)



It also contained peaks associated with ion fragments $-(\text{CH}_2)_n\text{CO}_2\text{CH}_3$ [87(11)]; $\text{C}_n\text{H}_{2n+1}$ [57(23), 71(72), 85(13)]; $\text{C}_n\text{H}_{2n-1}$ [55(100), 69(39), 83(18), 97(22), 111(22)]; $\text{C}_n\text{H}_{2n-3}$ [67(22), 81(19), 109(9)]; $\text{CH}=\text{C}-\text{OCH}_3$ [74(14)], and the following additional peaks: 252(4), 183(8), 121(11), 114(39), 112(11), 79(14), 59(24).

1.2(b) Attempted esterification of 9,12-Dioxo-octadecanoic acid with hot methanolic sulphuric acid

9,12-Dioxo-octadecanoic acid (100 mg, 0.32 mmol) and stearic acid (10 mg) as an internal standard were refluxed for 1 h with methanolic sulphuric acid (2% w/v, 10 ml). Usual work up yielded a product (104 mg) whose TLC indicated that its composition was similar to the product obtained in the attempted esterification of the furanoid acid with methanolic sulphuric acid, and with the following GLC (SP 2300, 240°) results.

* These ions arise from McLafferty rearrangement.

<u>ECL^a</u>	<u>Area^b (Weight %)</u>
18.0	11
20.7	45 (51)
21.1	2 (2)
24.3	2 (2)
29.0	40 (45)

a When chromatographed on a SP2340 column, the peaks of ECL 20.7 and 29.0 had values of 22.4 and 33.8 respectively.

b Figures in parenthesis give the relative areas of the reaction products after correction has been made for the internal standard.

1.3 Boron trifluoride in methanol

A mixture of the furanoid acid (27 mg), 0.09 mmol) and stearic acid (3 mg) was refluxed for 20 min. with methanol (3 ml) containing boron trifluoride - methanol complex (14%, 0.5 ml). The product (25 mg) was isolated in the usual way and examined by GLC (SP2340, 230°) with the following results.

<u>ECL</u>	<u>Area (Weight %)</u>
18.0	9
22.4	62 (68)
33.9	29 (32)

1.4 Acid wash at room temperature

A methanolic solution of the furanoid methyl ester (40 mg) and methyl stearate (10 mg) was acidified with dilute sulphuric acid (0.1 N, 5 ml) and the product

(46 mg) extracted with ether in the usual way with the following GLC (SP 2300, 240°) results.

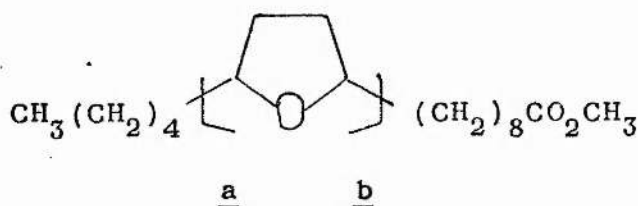
<u>ECL</u>	<u>Area (Weight %)</u>
18.0	19
20.7	72 (89)
22.6	9 (11)

2. HYDROGENATION

2.1 Hydrogenation of the furan moiety

The furanoid methyl ester (10 mg, 0.03 mmol) was hydrogenated in the presence of palladized charcoal (10%, 5 mg) in methanol (2 ml) at room temperature for 45 min. TLC of the product (8 mg) showed a single spot which was slightly more polar than the original furanoid ester and GLC showed a double peak of ECL 20.5 (75%) and 20.8 (25%). Its IR spectrum did not contain any of the absorption peak characteristic of long-chain furanoid methyl esters.

The mass spectrum displayed peaks at 312 (M,1), 281 (M-31,1), 241 (a,40), 223 (a-18,5), 209 (a-32,32) 191 (a-50,5), 141 (b,100) and 123 (b-18,57).



It also contained peaks associated with ion fragments $-(\text{CH}_2)_n\text{CO}_2\text{CH}_3$ [87(10)], $\text{C}_n\text{H}_{2n+1}$ [57(19), 71(19), 85(8), 99(5)], $\text{C}_n\text{H}_{2n-1}$ [55(97), 69(40), 83(23), 97(16), 111(6)], $\text{C}_n\text{H}_{2n-3}$ [67(62), 95(22), 109(7)], $\text{CH}_2=\overset{\text{OH}}{\text{C}}-\text{OCH}_3$

[74(57)], and the following additional peaks: 214(7), 191(5), 173(9).

2.2 Selective hydrogenation of methyl oleate in the presence of furanoid esters.

(a) In the presence of natural furanoid esters

A methanolic solution of a mixture of furanoid fatty acid methyl esters (25 mg) isolated from dogfish liver oil (fraction 4, p.208), was stirred with Adams catalyst (platinum oxide, 25 mg) under a hydrogen atmosphere. The reaction was stopped after 20 min. and GLC (DEGS) of the product (22 mg) showed the following results*.

<u>Reactant</u>		<u>Product</u>	
<u>ECL</u>	<u>% Area</u>	<u>ECL</u>	<u>% Area</u>
14.2	2.5	14.2	3.1
		16.3	2.2
17.0	2.5	17.0	3.1
		18.0	21.8
18.4	21.2	18.3	4.7
18.7	4.0		
20.3	4.2	20.3	4.4
20.7	2.7		
21.2	11.9	21.3	18.2
21.7	4.4	21.8	2.1
22.2	12.5	22.3	11.3
23.0	7.6	23.1	6.1
23.6	20.7	23.7	18.3

* Only the major peaks are recorded

(b) In the presence of synthetic furanoid esters

A methanolic solution of methyl 10,13-epoxyoctadeca-10,12-dienoate (20 mg) and methyl oleate (5 mg) was stirred

with palladium charcoal (10%, 10 mg) for 20 min. under a hydrogen atmosphere. GLC of the product showed a peak of ECL 18.0 (27%) and a double peak of ECL 20.5 (54%) and 20.8 (19%).

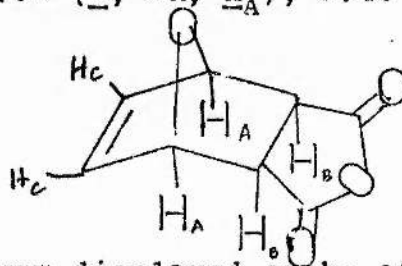
Use of Adams catalyst too resulted in considerable hydrogenation of the furan.

3. ATTEMPTED DIELS-ALDER REACTION

3.1 Adduction of furan with maleic anhydride

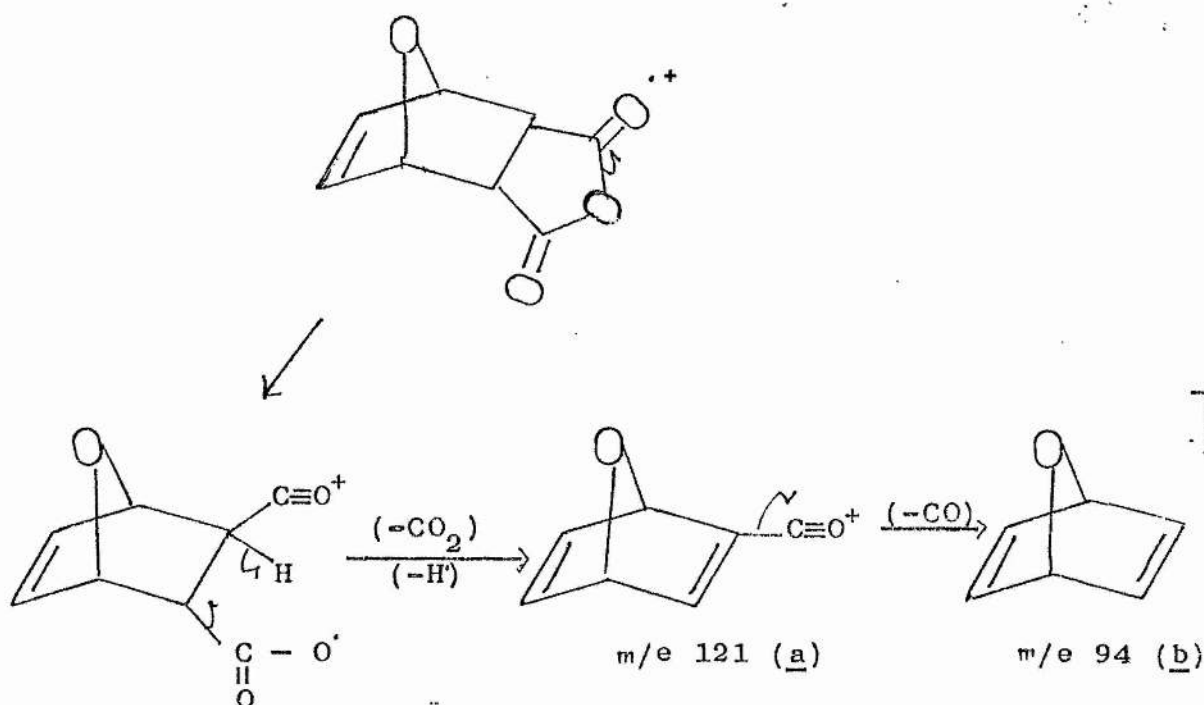
A solution of furan (1.36 g, 0.02 mol) and freshly prepared maleic anhydride (1.96 g, 0.02 mol) was refluxed with benzene (25 ml) for 2 hr. White needles [1.55 g, m.p. 116-117°C, Lit. 125-126⁰³³⁴] separated from solution after sitting at room temperature for 48 hrs. A second crop (0.87 g, m.p. 108-109°C) was obtained by cooling the filtrate at 0°C.

The PMR spectrum of the material of m.p. 111-112°C contained signals at 3.38 (s, 2H, $\underline{H_A}$), 5.40 (s, 2H, $\underline{H_B}$) and 6.67 (s, 2H, $\underline{H_C}$).



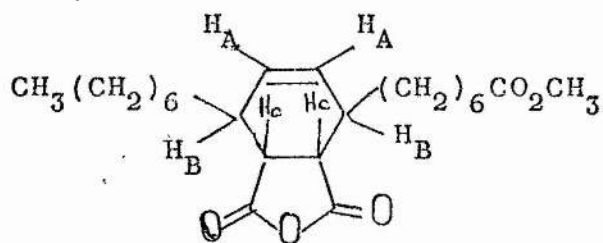
The mass spectrum displayed peaks at 121 (\underline{a} , 2) and 94 (\underline{b} , 100).

It also contained peaks at 68(23), 67(100), 65(96), 64(30), 55(18) and 54(24).

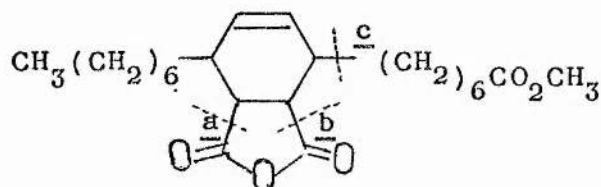


3.2 Adduction of methyl octadeca-trans-8,trans-10-dienoate with maleic anhydride.

Methyl octadeca-trans-8,trans-10dienoate (210 mg, 0.71 mmol) and freshly prepared maleic anhydride (70 mg, 0.71 mmol) were refluxed with benzene (10 ml) for 4 hrs. After removing the solvent under reduced pressure the residue was dissolved in ether (50 ml) and washed with water (3 x 25 ml). Finally, evaporation of ether under reduced pressure yielded a product (262 mg) whose PMR spectrum contained signals at 0.90 (t, 3H, CH_3CH_2-), 1.3-1.9 (m, 22H, $-\text{CH}_2\text{CH}_2\text{CH}_2-$), ~ 2.24 (m, 4H, $-\text{CH}_2\text{CH}_2\text{CO}_2\text{CH}_3$ and H_B), ~ 3.32 (m, 2H, H_C), 3.60 (s, 3H, $-\text{CO}_2\text{CH}_3$) and 5.78 (s, 2H, H_A).



The mass spectrum displayed peaks at 361 (M-31,1), 320 (a+b,8), 247 (a+b+c,7).



It also contained peaks associated with the ion fragments $-(CH_2)_nCO_2CH_3$ [87(30)], C_nH_{2n+1} [57(66)], C_nH_{2n-1} [55(100), 69(55), 83(33), 97(19)], C_nH_{2n-3} [67(52), 81(36), 95(22)], CH_2COCH_3 [74(30)], and the following additional peaks: 79(48), 77(20), 59(27).

3.3 Attempted addition of 10,13-furan acid with maleic anhydride.

(a) 10,13-furan acid (588 mg, 2.00 mmol) and freshly prepared maleic anhydride (196 mg, 2.00 mmol) were refluxed with benzene. TLC of the reaction mixture did not show a significant product even after 9 hrs of reaction time.

(b) The solvent was replaced by toluene and heating continued for a further 8 hrs, whereupon the material was recovered as described in the preceding section. PMR and mass spectral analysis showed that the material was unchanged furanoid acid.

(c) In a separate experiment, the reaction was

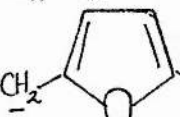
attempted on the furanoid methyl ester in both benzene and toluene but without success.

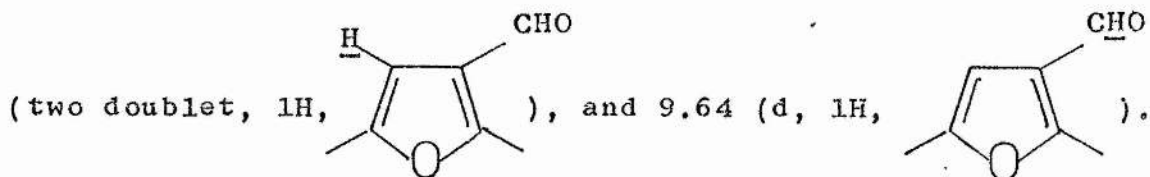
4. FORMYLATION - VILSMEIER REACTION

Phosphoryl chloride (154 mg, 1.00 mmol) was added dropwise to dimethylformamide (1 ml) at 10-20°, with stirring. The furanoid methyl ester in dimethylformamide (2 ml) was added to this solution maintaining the temperature at 10-20°. The reaction mixture was kept at room temperature (20°) for 64 hrs, whereupon it was poured into iced water (10 ml) and neutralized with sodium bicarbonate. Extraction with ether yielded the organic product (147 mg) whose GLC (SP 2300, 240°) showed peaks of ECL 25.8 (28%), 28.0 (60%) and several other late eluting peaks. The reaction product was separated into fractions: A (12 mg, 9%), B (53 mg, 42%), C (31 mg, 25%) and D (30 mg, 24%) by Prep. TLC (PE20).

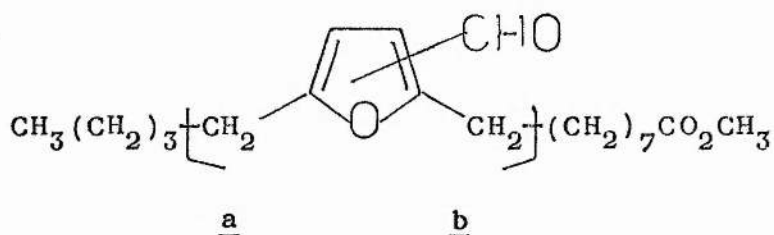
Fraction B

GLC of fraction B showed a single peak of ECL 25.8 and its IR spectrum showed peaks at 3440 (b,m), 3100 (b,w), 2700 (f,w), 1790 (f,w), 1630 (b,w), 1610 (f,m), 1550 (f,m), 1505 (f,w), 1010 (f,s) and 780 cm⁻¹ (f,s), in addition to the usual absorptions. The PMR spectrum contained signals at 0.90 (t, 3H, CH₃CH₂-), 1.30-1.8 (m, H, -CH₂CH₂CH₂-), 2.22 (t, 2H, -CH₂CH₂CO₂CH₃), 2.58

(t, 4H, -CH₂-, 3.60 (s, 3H, -CO₂CH₃), 5.9 and 6.0



The mass spectrum of fraction B displayed peaks at 336 (M, 14), 308 ($\underline{c}+1$, 40), 307 (\underline{c} , 95), 251 ($\underline{a}-28$, 4), 247 ($\underline{a}-32$, 4), 193 ($\underline{b}+14$, 9), 180 ($\underline{b}+1$, 30), 179 (\underline{b} , 12), 165 ($\underline{b}+14-28$), 151 ($\underline{b}-28$, 100), 123 ($\underline{a}+\underline{b}$, 47), 95 ($\underline{a}+\underline{b}-28$, 62).

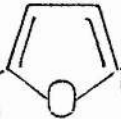


It also contained peaks associated with ion fragments $-(\text{CH}_2)_n\text{CO}_2\text{CH}_3$ [87(21)], $\text{C}_n\text{H}_{2n+1}$ [57(55), 71(37), 85(19), 99(37)], $\text{C}_n\text{H}_{2n-1}$ [55(100), 69(56), 83(20), 97(20), 111(11), 125(11), 139(9), 153(20), 167(22)], $\text{C}_n\text{H}_{2n-3}$ [67(37), 81(50), 109(22), 137(20)], $\text{CH}_2=\text{C}-\text{OCH}_3$ [74(54)], and the following additional peaks: 275(7), 207(16), 149(13), 135(15), 121(24), 107(77), 91(15), 79(19), 77(15), 59(25), 53(19).

Fraction C

GLC of this fraction showed peaks of ECL 25.8 (6%), 28.0 (76%), and 30.8 (18%). Its IR spectrum had peaks at 3460 (b,m), 1670 (f,s), 1580 (f,w), 1510 (f,s), 1010 (f,m), 785 cm^{-1} (f,s), in addition to the usual absorptions. The PMR spectrum contained signals at 0.92 (t, 3H, CH_3CH_2-),

1.32-1.8 (m, 18H, $-\text{CH}_2\text{CH}_2\text{CH}_2-$), 2.22 (t, 2H, $-\text{CH}_2\text{CH}_2\text{CO}_2\text{CH}_3$),

2.68 (t, 4H, CH_2CH_2  CH_2CH_2), 3.58 (s, 3H, $-\text{CO}_2\text{CH}_3$).

Its mass spectrum contained peaks at 336(1), 322(8), 307(3), 291(8), 280(2), 248(4), 220(3), 207(52), 206(12), 193(22), 180(85), 179(14), 177(7), 167(7), 165(50), 163(4), 153(7), 151(12), 149(8), 139(13), 137(32), 136(12), 123(92), 121(10), 109(15), 107(12), 99(10), 97(15), 95(22), 87(15), 85(42), 83(21), 81(60), 79(15), 74(35), 71(37), 69(40), 67(28), 59(32), 57(95), 55(100), 53(30).

Attempted reduction of fraction B

Fraction B (50 mg, 0.15 mmol) and *p*-toluenesulphonylhydrazide (38 mg, 0.20 mmol) were refluxed gently with methanol (3 ml) for 3 hrs. The reaction mixture was allowed to cool to room temperature, sodium borohydride (120 mg, 3.20 mmol) was added in small portions over a period of 1 hr. and refluxed for a further 8 hrs. Methanol was removed under reduced pressure, replaced by ether (10 ml) and the solution was washed successively with water (3 ml), aqueous sodium bicarbonate (5%, 3 ml), dilute hydrochloric acid (2M, 3 ml), and finally with water (2 ml). Removal of the solvent gave a product (41 mg) whose IR spectrum contained peaks at 3180 (b,m, $-\text{N}-\text{H}$), 1590 (f,w), aromatic $\text{C}=\text{C}$, 1160 (f,s, $-\text{S}=\text{O}$), 810 (f,m, 1,4-disubstituted benzene ring).

5. ATTEMPTED BROMINATION

Bromine (80 mg, 0.50 mmol) was added dropwise to a stirred solution of the furanoid methyl ester (154 mg, 0.50 mmol) in carbondisulphide (5 ml) at 0-5°. The bromine was decolourized until almost all of the weighed quantity had been added (45 min.) and the colour persisted thereafter. After the addition was complete, the reaction mixture was poured into ice (10 g) and the organic layer was washed successively with sodium bicarbonate (5%, 5 ml) and water (5 ml). GLC (SP 2300, 240°) of the product (182 mg) showed a series of peaks with the peaks of ECL 22.0 (9%), 22.8 (59%), and 24.8 (14%) being dominant. On standing for 3-4 hrs, the reaction product deposited a brownish precipitate and GLC of the total material showed that the component of ECL 22.8 had been diminished. TLC of the reaction product showed streaks and it was not investigated further.

6. ATMOSPHERIC OXIDATION

(i) A mixture of F₁-F₇ and P₁-P₃ (isolated from dogfish liver oil was stored in petrol at -20°C for 10 months. The material streaked on TLC plates and the relative proportions of P and F esters obtained by GLC were as follows

<u>Composition</u>	<u>Fresh Extract</u>	<u>10 Month Old Extract</u>
P ₁₆	9.6	19.7
P ₁₉	4.6	9.8
P ₂₀	14.9	34.4
F ₁	1.5	1.1
F ₂	4.8	4.7
F ₃	2.8	1.8
F ₄	12.6	8.5
F ₅	4.7	4.9
F ₆	42.6	14.2
F ₇	1.9	0.9

(ii) A mixture of F₁-F₆ and P₁-P₃ were exposed to a gentle stream of oxygen and the material examined periodically by TLC and GLC. TLC indicated formation of increasing quantities of polar products with time. The relative proportions of F and P esters obtained by GLC are given below.

<u>Composition</u>	<u>Start (0 Day)</u>	<u>2 Days</u>	<u>14 Days</u>	<u>21 Days</u>
P ₁₆	8.6	9.7	14.3	16.6
P ₁₉	14.5	15.8	22.7	25.0
P ₂₀	32.8	34.3	46.8	55.7
F ₁	0.8	0.6	0.6	-
F ₂	4.4	3.5	3.8	1.4
F ₃	1.8	1.5	0.4	-
F ₄	6.2	5.4	1.7	-
F ₅	3.0	2.6	2.7	0.8
F ₆	27.9	26.6	7.0	0.5

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Lectures

1. "Furanoid Fatty Acids".
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2. "Synthesis of Fatty Acids Containing a Furan-ring".
F.D. Gunstone and R.C. Wijesundera, presented to
the 14th World Congress of the International Society
for Fat Research, Brighton, England, September, 1978.